


FORM PTO-1390 (REV 11-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 2590-30
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) <div style="font-size: 2em; font-weight: bold; display: inline-block;">09/806831</div> Unknown
INTERNATIONAL APPLICATION NO. PCT/CH99/00476	INTERNATIONAL FILING DATE 7 October 1999	PRIORITY DATE CLAIMED 7 October 1998
TITLE OF INVENTION METHOD AND APPARATUS FOR MEASURING LOCALLY AND SUPERFICIALLY THE SCATTERING AND ABSORPTION PROPERTIES OF TURBID MEDIA		
APPLICANT(S) FOR DO/EO/US BEVILACQUA et al		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input checked="" type="checkbox"/> The U.S. has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <div style="margin-left: 20px;"> <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). <input checked="" type="checkbox"/> has been communicated by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). </div> 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <div style="margin-left: 20px;"> <input type="checkbox"/> is attached hereto. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). </div> 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <div style="margin-left: 20px;"> <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). <input type="checkbox"/> have been communicated by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input type="checkbox"/> have not been made and will not be made. </div> 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).		
Items 11 To 20 below concern document(s) or information included:		
11. <input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98. 12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825. 18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. <input checked="" type="checkbox"/> Other items or information. PTO-1449 and copy of International Search Report		

U.S. APPLICATION NO. 09/806831 Unknown		INTERNATIONAL APPLICATION NO. PCT/CH99/00476		ATTORNEY'S DOCKET NUMBER 2590-30	
21. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5): -- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO\$1000.00 -- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....\$860.00 -- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO\$710.00 -- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4).....\$690.00 -- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....\$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				<div style="text-align: right;">\$ 860.00</div>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).				\$ 0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	20	-20 = 0	X \$18.00	\$ 0.00	
Independent Claims	1	-3 = 0	X \$80.00	\$ 0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			\$270.00	\$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 860.00	
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				430.00	
SUBTOTAL =				\$ 430.00	
Processing fee of \$130.00, for furnishing the English Translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)).				0.00	
TOTAL NATIONAL FEE =				\$ 430.00	
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). \$40.00 per property				40.00	
Fee for Petition to Revive Unintentionally Abandoned Application (\$1240.00 - Small Entity = \$620.00)				0.00	
TOTAL FEES ENCLOSED =				\$ 470.00	
				Amount to be:	
				refunded \$	
				Charged \$	
a. <input checked="" type="checkbox"/> A check in the amount of \$470.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 14-1140 in the amount of \$_____ to cover the above fees. A duplicate copy of this form is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed. d. <input checked="" type="checkbox"/> The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.					
NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) -or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: NIXON & VANDERHYE P.C. 1100 North Glebe Road, 8 th Floor Arlington, Virginia 22201-4714 Telephone: (703) 816-4000					
 SIGNATURE					
Michael J. Shea NAME					
34,725 REGISTRATION NUMBER					
April 5, 2001 Date					

09/806831

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

BEVILACQUA et al

Atty. Ref.: **2590-30**

Serial No. **Unknown**

Group:

National Phase of: **PCT/CH99/00476**

International Filing Date: **7 October 1999**

Filed: **April 5, 2001**

Examiner:

For: **METHOD AND APPARATUS FOR MEASURING
LOCALLY AND SUPERFICIALLY THE SCATTERING
AND ABSORPTION PROPERTIES OF TURBID MEDIA**

* * * * *

April 5, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend the claims as follows:

IN THE SPECIFICATION

On page 1, after the title, please insert --This application is a 371 of PCT/CH99/00476.--

IN THE CLAIMS (AS AMENDED)

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

1. (Amended) A method for local and superficial characterization of a turbid medium using the following parameters:

- 1) the refractive index n of the turbid medium
- 2) the absorption coefficient μ_a of the turbid medium
- 3) the reduced scattering coefficient μ_s' of the turbid medium

4) the phase function parameter $\gamma = (1 - g_2)/(1 - g_1)$ of the turbid media, where g_1 and g_2 are the first two moments of the Legendre polynomial development of the phase function p ,

and comprising the steps of:

- measuring the spatially-resolved reflectance $R(\rho)$ of the turbid medium (ρ being the source-detector distance) by any means, comprising an illumination beam as a source and an optical detector, which, by using optional signal processing, which may involve filtering and de-convolution operation to correct for the non-zero area of either the illumination source or the detector, allows for the precise determination of the said spatially-resolved reflectance $R(\rho)$,
- mathematically processing $R(\rho)$ to compute at least one of the said parameters: n, μ_a, μ_s', γ and/or the variations, in time and/or space, of at least one of the said parameters: $\Delta n, \Delta \mu_a, \Delta \mu_s', \Delta \gamma$, whereby an “inverse problem”, which consists in extracting the optical coefficients from the spatially resolved reflectance data is solved, and whereby a “direct problem” consists in computing the spatially resolved reflectance from the values of the optical coefficients n, μ_a, μ_s', γ involved in a model of propagation of the light in turbid medium and whereby the said “model” incorporates a Legendre polynomial development to the second order of the said “phase function”, and whereby the said “phase parameter” γ is introduced in the computation as an independent parameter.

2. The method of claim 1, wherein said spatially resolved reflectance is measured by a probe comprising at least one optical fiber carrying the light from the source unit to the turbid medium and at least one optical fiber collecting the reflected light and carrying it to detection unit, whereby the combination of a variety of emitting fibers and of receiving fibers yields a set of distances ρ at which the reflectance $R(\rho)$ is measured.

3. (Amended) The method according to claim 1, wherein said spatially resolved reflectance $R(\rho)$ is measured for a set of values of ρ , by using a probe composed of optical fibers in any of the following configurations:

- one emitting optical fiber and a set of optical receiving fibers
- a set of optical emitting fibers and one optical receiving fiber
- a set of optical emitting fibers and a set of optical receiving fibers

giving the spatially resolved reflectance $R(\rho)$ at a variety of source-detector distances ρ and wherein the emitting and receiving fibers are arranged in one of the following configurations:

- on a line,
- on crossed lines,
- on a circle,
- on an ellipse
- on crossed ellipses
- on a disk, a rectangle, or any surface, as a dense and contiguous arrangement of fibers,
- on any pattern resulting from the combination of the above mentioned patterns.

4. (Amended) The method according to claim 3, wherein said spatially resolved reflectance is measured by an optical and electronic micro-system comprising a collimated or focused beam as illuminating source and 1D or 2D arrays of optical detectors.

5. (Amended) The method according to claim 4, wherein either the probe or the optical and electronic micro-system are put in contact to the turbid medium.

6. (Amended) The method of claim 1, wherein said spatially resolved reflectance is measured by a non-contact system, comprising at least one of the following combination of optical systems:

- a fixed optical system to irradiate the turbid medium with a collimated or focused beam forming the illuminating source and a fixed optical system comprising an imaging system forming the image of the measured area of the turbid medium on a said “optical detector”, which can be formed of 1D or 2D array of optical detectors, whereby this second optical system can be identical to the first one and whereby the array of optical detectors can be either one of the following systems:
 - a set of optical fibers,
 - an optical and electronic micro-system (MOEM),
- a fixed optical system for the collimated beam illuminating source and a scanned optical system for the said “optical detector”,
- a scanning optical system for the collimated beam illuminating source and a fixed optical system for the said “optical detector”,
- a scanning optical system for the collimated or focused beam used as an illuminating source and a scanning optical system for the said “optical detector”.

7. (Amended) The method according to claim 1, wherein the absorption coefficient μ_a , the reduced scattering coefficient μ_s' and the phase function parameter γ are determined by fitting the measured spatially-resolved reflectance $R(\rho, \mu_s', \mu_a, \gamma)$ to a set of discretized data obtained by using Monte Carlo simulations, or to interpolating functions giving a

continuous approximation of the discretized data obtained by Monte Carlo simulations, and whereby said “Monte Carlo simulations” are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

8. (Amended) The method according to claim 7, wherein one or more of the following signal processing steps are performed:

- fitting the measured reflectance $R(\rho)$ by the function:

$$m_1 \rho^{m_2} \exp(m_3 \rho)$$

to give the values of the parameters m_1 , m_2 and m_3 , assuming that the expression

$R(\rho) = m_1 \rho^{m_2} \exp(m_3 \rho)$ gives a smoothed expression of the spatially resolved reflectance $R(\rho)$,

- computing the slopes $\frac{\partial}{\partial \rho} \sqrt{R(\rho)}$ and $\frac{\partial}{\partial \rho} (\ln R(\rho))$, or any mathematical combinations

of these two latter quantities and $R(\rho)$, from analytical functions using the parameters m_1 , m_2 , m_3 ,

or by numerical procedures from the expression $R(\rho) = m_1 \rho^{m_2} \exp(m_3 \rho)$,

- computing the values of at least one of the said parameters: n , μ_a , μ_s' , γ and/or the variations, in time and/or space, of at least one of the said parameters: Δn , $\Delta \mu_a$, $\Delta \mu_s'$, $\Delta \gamma$ from the relationship between the reflectance intensity $R(\rho)$ and the slope of $\ln R(\rho)$ (denoted $\partial \rho \ln R$), determined at a fixed distance ρ comparable to the transport mean free path, whereby the computation is made from the data obtained by Monte Carlo simulations, provided that said “Monte Carlo simulations” are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

9. (Amended) The method according to claim 7, wherein the following signal processing steps are performed:

- computing the reduced scattering coefficient μ_s' and the phase function parameter γ by using the following form of the reflectance:

$$R(\rho) = (A(\rho, \gamma, \mu_s') + B(\mu_a, \mu_s'))^2$$

where the function $A(\rho, \gamma, \mu_s')$ and $B(\mu_a, \mu_s')$ depend also on the sources and detectors characteristics, and the refractive index of the sample, and comprising the steps of:

- computing the slopes of the square root of the spatially resolved reflectance $\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s', \mu_a, \gamma)} = \frac{\partial A}{\partial \rho}(\rho, \mu_s', \gamma)$, which depends weakly on the absorption coefficient μ_a for $0.3 < \rho \mu_s' < 5$, for at least two distances ρ ,
- determining the parameters μ_s' and γ by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

10. (Amended) The method of claim 9, wherein the absorption coefficient μ_a is determined by using the equation:

$$\mu_a = h \left[\sqrt{R(\rho, \mu_s', \mu_a, \gamma)} - f(\gamma, \mu_s') \right]$$

where f and h are continuous functions of the parameters ρ , μ_s' and γ that can be approximated by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said “Monte Carlo simulations” are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

11. (Amended) The method according to claim 1, wherein the difference $\Delta\mu_a = \mu_a - \mu_{a0}$ between the absorption coefficient μ_a and a known value μ_{a0} is computed from the quantity $\sqrt{R(\rho, \mu_s', \mu_{a0}, \gamma)} - \sqrt{R(\rho, \mu_s', \mu_a, \gamma)} = B(a') - B(a_0')$, whereby the function $B(a')$ of the albedo a' can be approximated by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said “Monte Carlo simulations” are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order, and whereby the calculation can be done for a single γ value, because the influence of the phase function parameter and γ in $B(a')$ are particularly weak.

12. (Amended) The method according to claim 1, wherein the illuminating source is a broadband source or a white light source and the detector unit comprises a spectrograph or any wavelength analysis system to measure the wavelength dependence of at least one of the parameters $(n, \mu_a, \mu_s', \gamma)$.

13. (Amended) The method according to claim 1, wherein said turbid medium is a biological medium.

14. (Amended) The method according to claim 1, wherein the measurement and processing is repeated at different locations of the sample, to build images of any one of the said parameters $(n, \mu_a, \mu_s', \gamma)$.

15. (Amended) An apparatus using the method of claim 1 for local and superficial characterization of a turbid medium,

- a) comprising a source, a detection unit, reference means, signal processing means, a probe comprising at least one optical fiber connecting said source unit to the turbid medium and at least one optical fiber connecting the turbid medium to the said detection unit, and reference means
- b) where the distance between the source and the detector is close to one transport mean free path.

16. (Amended) An apparatus using the method of claim 1 for local and superficial characterization of a turbid medium,

- a) comprising an optical and electronic micro-system comprising at least one illuminating source, at least one detector, signal processing means and reference means,
- b) where the distance between the source and the detector is close to one transport mean free path

17. (Amended) An apparatus using the method of claim 1 for local and superficial characterization of a turbid medium,

- a) comprising a collimated or focused beam used as an illuminating source, at least an optical detector for the detection unit, a fixed or scanning optical system for the illuminating source and

a fixed or scanning optical system for the said “optical detector”, signal processing means and reference means,

b) where the distance between the source and the detector is close to one transport mean free path

18. (Amended) A system comprising three apparatus described in claim 15, and characterized by the fact that the distance between the collimated or focused optical beam used as illuminating source and the emitting point connected to an optical detector varies from 0.1 to 2mm. for application to biological media and to turbid media having a transport mean free path similar to biological media.

19. (Amended) A test, where the control of the homogeneity of the sample over the probed area is performed with the apparatus of 15, which can be carried out according to the following procedure: in the apparatus, disposing two illuminating fibers symmetrically in regard to the collecting fibers; comparing the reflectance curves for each illuminating fiber to detect the heterogeneity of the investigated region or obstructions beneath the fibers; and, if the two curves are sufficiently close, validating the measurement and calculating the average of the two curves.

20. (Amended) A calibration and normalization procedure, which are carried out with the apparatus of claim 15, whereby the following steps are performed:

1) in order to perform relative intensity measurements, the differences of transmitted intensity between each fiber for the apparatus of claim 15 are determined by performing a measurement on a turbid phantom illuminated uniformly or a diffusing sphere of perfectly uniform properties;

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2) in order to perform absolute intensity measurements, a calibration performed on a turbid medium of known optical properties, which can be realized according to any one of the following recipes:

- a) solid or liquid turbid medium which properties have been measured by other techniques, or reported in the literature,
- b) water suspension of micro-spheres of known size distribution and refractive index.

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,

NIXON & VANDERHYE P.C.

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Amended) A method for local and superficial [(probed volume and measuring area with dimensions on the order of one transport mean free path)] characterization of a turbid medium using the following parameters:

- 1) the refractive index n of the turbid medium
- 2) the absorption coefficient μ_a of the turbid medium
- 3) the reduced scattering coefficient μ_s' of the turbid medium
- 4) the phase function parameter $\gamma = (1-g_2)/(1-g_1)$ of the turbid media, where g_1 and g_2 are the first two moments of the Legendre polynomial development of the phase function p , and comprising the steps of:

- measuring the spatially-resolved reflectance $R(\rho)$ of the turbid medium (ρ being the source-detector distance) by any means [mean], comprising an illumination beam as a source and an optical detector, which, by using optional signal processing, which may involve filtering and deconvolution operation to correct for the non-zero area of either the illumination source or the detector, allows for the precise determination of the said spatially-resolved reflectance $R(\rho)$,
- mathematically processing $R(\rho)$ to compute at least one of the said parameters: n , μ_a , μ_s' , γ and/or the variations, in time and/or space, of at least one of the said parameters: Δn , $\Delta\mu_a$, $\Delta\mu_s'$, $\Delta\gamma$, whereby an [the said] “inverse problem”, which consists in extracting the optical coefficients from the spatially resolved reflectance data is solved, and whereby a [the said] “direct problem” consists in computing the spatially resolved reflectance from the values of the optical coefficients n , μ_a , μ_s' , γ involved in a model of propagation of the light in turbid medium and whereby the said “model” incorporates a Legendre polynomial development to the second

order of the said “phase function”, and whereby the said “phase parameter” γ is introduced in the computation as an independent parameter.

2. The method of claim 1, wherein said spatially resolved reflectance is measured by a probe comprising at least one optical fiber carrying the light from the source unit to the turbid medium and at least one optical fiber collecting the reflected light and carrying it to detection unit, whereby the combination of a variety of emitting fibers and of receiving fibers yields a set of distances ρ at which the reflectance $R(\rho)$ is measured.

3. (Amended) The method according to claim [any one of claims] 1 [and 2], wherein said spatially resolved reflectance $R(\rho)$ is measured for a set of values of ρ , by using a probe composed of optical fibers in any of the following configurations:

- one emitting optical fiber and a set of optical receiving fibers
- a set of optical emitting fibers and one optical receiving fiber
- a set of optical emitting fibers and a set of optical receiving fibers

giving the spatially resolved reflectance $R(\rho)$ at a variety of source–detector distances [distance] ρ and wherein the [. The] emitting and receiving fibers are [can be] arranged in one of the following [a variety of] configurations[. In some particular arrangement they can be positioned]:

- on a line,
- on crossed lines,
- on a circle,
- on an ellipse
- on crossed ellipses

- on a disk, a rectangle, or any surface, as a dense and contiguous arrangement of fibers[, such as it can be obtained with a bundle of fibers or a multi-core fiber],
- on any pattern resulting from the combination of the above mentioned patterns.

4. (Amended) The method according to claim [any one of claims 1, 2 and] 3, wherein said spatially resolved reflectance is measured by an optical and electronic micro-system comprising a collimated or focused beam as illuminating source and 1D or 2D arrays of optical detectors[, arranged in a way similar to the one described in claim 3, and which, in some embodiment, can be a CCD or MOS camera].

5. (Amended) The method according to claim [any one of claims 1-]4, wherein either [where] the probe [of claims 2 and 3] or the optical and electronic micro-system [of claim 4] are put in contact to the turbid medium.

6. (Amended) The method of claim 1, wherein said spatially resolved reflectance is measured by a non-contact system, comprising at least one of the following combination of optical systems:

- a fixed optical system to irradiate the turbid medium with a collimated or focused beam forming the illuminating source and a fixed optical system comprising an imaging system forming the image of the measured area of the turbid medium on a said “optical detector”, which can be formed of 1D or 2D array of optical detectors, whereby this second optical system can be identical to the first one and whereby the array of optical detectors can be either one of the following systems [system]:

- a set of optical fibers [arranged in a variety of configurations similar to those described in claim 3],
- an optical and electronic micro-system (MOEM)[, which in some embodiment can be a CCD or MOS camera, with a functionality similar to the one described in claim 4],
- a fixed optical system for the collimated beam illuminating source and a scanned optical system for the said “optical detector”,
- a scanning optical system for the collimated beam illuminating source and a fixed optical system for the said “optical detector”,
- a scanning optical system for the collimated or focused beam used as an illuminating source and a scanning optical system for the said “optical detector”.

7. (Amended) The method according to claim [any one of claims] 1[. to 6.], wherein the absorption coefficient μ_a , the reduced scattering coefficient μ_s' and the phase function parameter γ are determined by fitting the measured spatially-resolved reflectance $R(\rho, \mu_s', \mu_a, \gamma)$ to a set of discretized data obtained by using Monte Carlo simulations, or to interpolating functions giving a continuous approximation of the discretized data obtained by Monte Carlo simulations, and whereby said “Monte Carlo simulations” are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order[In a preferred embodiment, cubic splines are used as interpolating functions].

8. (Amended) The method according to claim [any one of claims 1 to] 7, wherein one or more of the following signal processing steps are [can be] performed [in addition to the

processing steps of any of the claims 1-6 whereby the processing of the spatially resolved reflectance data of claim 7 can be simplified and accelerated by]:

- fitting the measured reflectance $R(\rho)$ by the function:

$$m_1 \rho^{m_2} \exp(m_3 \rho)$$

to give [This fit gives] the values of the parameters m_1 , m_2 and m_3 , assuming that the expression $R(\rho) = m_1 \rho^{m_2} \exp(m_3 \rho)$ gives a smoothed expression of the spatially resolved reflectance $R(\rho)$].

- computing [Computing] the slopes $\frac{\partial}{\partial \rho} \sqrt{R(\rho)}$ and $\frac{\partial}{\partial \rho} (\ln R(\rho))$, or any mathematical combinations of these two latter quantities and $R(\rho)$, from analytical functions using the parameters m_1 , m_2 , m_3 , or by numerical procedures from the expression $R(\rho) = m_1 \rho^{m_2} \exp(m_3 \rho)$.
- computing the values of at least one of the said parameters: n , μ_a , μ_s , γ and/or the variations, in time and/or space, of at least one of the said parameters: Δn , $\Delta \mu_a$, $\Delta \mu_s$, $\Delta \gamma$ from the relationship between the reflectance intensity $R(\rho)$ and the slope of $\ln R(\rho)$ (denoted $\partial \rho \ln R$), determined at a fixed distance ρ comparable to the transport mean free path, whereby the computation is made from the data obtained by Monte Carlo simulations, provided that said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

9. (Amended) The method according to claim 7 [any one of claims 1 to 7], wherein the following signal processing steps are [can be] performed [in addition to the processing steps of any claim 1-6 whereby the processing of the spatially resolved reflectance data of claim 7 can be simplified and accelerated by]:

- computing the reduced scattering coefficient μ_s' and the phase function parameter γ by using the following form of the reflectance:

$$R(\rho) = (A(\rho, \gamma, \mu_s') + B(\mu_a, \mu_s'))^2$$

where the function $A(\rho, \gamma, \mu_s')$ and $B(\mu_a, \mu_s')$ depend also on the sources and detectors characteristics, and the refractive index of the sample, and comprising the steps of:

- computing [Computing] the slopes of the square root of the spatially resolved reflectance

$$\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s', \mu_a, \gamma)} = \frac{\partial A}{\partial \rho}(\rho, \mu_s', \gamma), \text{ which depends weakly on the absorption coefficient } \mu_a \text{ for}$$

$0.3 < \rho \mu_s' < 5$, for at least two distances ρ ,

- determining [Determining] the parameters μ_s' and γ by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said “Monte Carlo simulations” are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

10. (Amended) The method of claim [claims] 9, wherein the absorption coefficient μ_a is determined by using the equation:

$$\mu_a = h \left[\sqrt{R(\rho, \mu_s', \mu_a, \gamma)} - f(\gamma, \mu_s') \right]$$

where f and h are continuous functions of the parameters ρ , μ_s' and γ [of] that can be approximated by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said “Monte Carlo simulations” are based on a photon propagation model comprising a

phase function approximated by a Legendre polynomial development limited to the second order.

11. (Amended) The method according to claim [any one of claims] 1 [to 7], wherein the difference $\Delta\mu_a = \mu_a - \mu_{a0}$ between the absorption coefficient μ_a and a known value μ_{a0} is computed from the quantity $\sqrt{R(\rho, \mu_s, \mu_{a0}, \gamma)} - \sqrt{R(\rho, \mu_s, \mu_a, \gamma)} = B(a') - B(a'_0)$, whereby the function $B(a')$ of the albedo a' can be approximated by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order, and whereby the calculation can be done for a single γ value, because the influence of the phase function parameter and γ in $B(a')$ are particularly weak.

12. (Amended) The method according to claim [any one of claims] 1 [to 11], wherein the illuminating source is a broadband source or a white light source and the detector unit comprises a spectrograph or any wavelength analysis system to measure the wavelength dependence of at least one of the parameters $(n, \mu_a, \mu_s, \gamma)$.

13. (Amended) The method according to claim [any one of claims] 1 [to 12], wherein said turbid medium is a biological medium.

14. (Amended) The method according to claim [any one of claims] 1 [to 13], wherein the measurement and processing is repeated at different locations [location] of the

sample [(multi-site measurements)], to build images of any one of the said parameters

$(n, \mu_a, \mu_s', \gamma)$.

15. (Amended) An apparatus using the method of claim 1 [to 3, 5 or 7 to 14] for local and superficial characterization of a turbid medium,
a) comprising a source, a detection unit, reference means, signal processing means, a probe comprising at least one optical fiber connecting said source unit to the turbid medium and at least one optical fiber connecting the turbid medium to the said detection unit, and reference means
b) where the distance between the source and the detector is close to one transport mean free path.

16. (Amended) An apparatus using the method of claim 1[, 4, 5, or 7 to 14] for local and superficial characterization of a turbid medium,
a) comprising an optical and electronic micro-system comprising at least one illuminating source, at least one detector, signal processing means and reference means,
b) where the distance between the source and the detector is close to one transport mean free path

17. (Amended) An apparatus using the method of claim 1 [or 6 to 14] for local and superficial characterization of a turbid medium,
a) comprising a collimated or focused beam used as an illuminating source, at least an optical detector for the detection unit, a fixed or scanning optical system for the illuminating source and

a fixed or scanning optical system for the said “optical detector”, signal processing means and reference means,

b) where the distance between the source and the detector is close to one transport mean free path

18. (Amended) A system comprising three [Three] apparatus [similar to the apparatus] described in claim [any one of the claims] 15[, 16 and 17], and characterized by the fact that the distance between the collimated or focused optical beam used as illuminating source and the emitting point connected to an optical detector varies from 0.1 to 2mm. for application to biological media and to turbid media having a transport mean free path similar to biological media.

19. (Amended) A test, where the control of the homogeneity of the sample over the probed area is performed with the apparatus of [any one of the claims] 15[, 16,17 and 18], which can be carried out according to the following [optional] procedure: in [In] the apparatus, disposing two illuminating fibers [are disposed] symmetrically in regard to the collecting fibers; comparing[. If the sample is homogeneous,] the reflectance curves for each [curve should be identical with either] illuminating fiber to detect the[. Therefore,] heterogeneity of the investigated region or obstructions beneath the fibers; and, if [are detected by comparing the two curves. If] the two curves are sufficiently close, validating the measurement [is validated] and calculating the average of the two curves [is calculated].

20. (Amended) A calibration and normalization procedure, which are carried out [optionally] with the apparatus of claim [any one of the claims] 15[, 16,17 and 18], whereby the following steps are performed:

1) in [In] order to perform relative intensity measurements, the differences of transmitted intensity between each fiber for the apparatus of claim 15 [or the differences of signal intensity between each optical detector for any of the claims 16 and 17,] are determined by performing a measurement on a turbid phantom illuminated uniformly or a diffusing sphere of perfectly uniform properties;[. In this calibration procedure, the background light, measured with the light source turned off, must be subtracted from the signal. The obtained values are used to multiply the measurements given by each fiber and/or detector to correct the relative intensity measurements.]

2) in [In] order to perform absolute intensity measurements, a calibration [can be] performed on a turbid medium of known optical properties, which can be realized according to any one of the following recipes [recipe]:

a) solid or liquid turbid medium which properties have been measured by other techniques, or reported in the literature,

b) water suspension of micro-spheres of known size distribution and refractive index[.

Absorbing dye may be added to the suspension. In this case, the scattering properties are calculated from Mie theory, and the absorption coefficient is assumed to be equal to the water absorption coefficient, if no absorbing dye is added. If an absorbing dye is used, the absorption coefficient can be measured by a spectrophotometer, before mixing the solution with any scattering materials.

[illegible]

METHOD AND APPARATUS FOR MEASURING LOCALLY AND SUPERFICIALLY THE SCATTERING AND ABSORPTION PROPERTIES OF TURBID MEDIA

1. Field of the invention

The present invention relates to a method and an apparatus to quantify the optical scattering and absorption properties of turbid media, that can be applied in the extended optical domain of electromagnetic waves from far infrared (up to the microwave domain) to the far UV domain, i.e. where the energies of the photons composing the electromagnetic waves range from a few milli-electron-Volt to more than ten electron-Volt. More precisely the present invention relates to a non-invasive measurement, over a small area of the sample surface. Local and superficial characterization of biological tissues in vivo is a major application of this invention.

2. Related Background Art.

Different techniques have already been proposed to quantitatively determine the absorption and reduced scattering coefficients of turbid media¹. Most of the non-invasive methods are based on the measurement of spatially and/or temporally-resolved reflectance. The principle is as follows: the turbid medium is illuminated by a collimated or focused light source. The backscattered light is measured by one or several detectors. Different types of measurements are possible, depending on the time-dependence of the illuminating source: steady-state (continuous source), time-domain (short pulsed source)

or frequency domain (amplitude modulated source). The present invention relates to the case of steady-state measurements, performed at different distances ρ between the source and the detectors. However, the technique presented here can be complemented by time- or frequency-domain measurements.

The range of ρ values is an important point to consider, when comparing different methods based on the measurement of the reflectance. First, the probed volume of the turbid medium is related to the source-detector separation ρ . The larger the source-detector separation, the deeper the average depth probed. Second, depending on the range of ρ , different mathematical processing must be used to obtain the optical properties from the raw data.

At least two cases must be distinguished.

1) The first case corresponds to source-detector separations larger than several transport mean free paths. For typical biological tissue optical properties², this case correspond to a source-detector separation larger than 2 mm. An analytical form of the reflectance can be obtained from the diffusion equation, if the absorption coefficient μ_a is sufficiently lower than the scattering coefficient μ_s (typically ten times). In such a case, the relevant optical properties are the refractive index, the absorption coefficient and the reduced scattering coefficient. The average depth of probing is on the same order than the source-detector separation ρ .

Such methods have been already published, and are the object of patents (Ref., Patent 5,517,987 Tsuchiya, Patent 5,676,142 Miwa et al.).

2) The second case corresponds to source-detector separations close to one transport mean free path. For biological tissues², such source-detector separations correspond typically to distances from 0.1 to 2 mm. The average depth probed is on the order of 1 mm. Such small source-detector separations enable the measurement of the optical properties of a small tissue volume.

Wang et al. (Patent 5,630,423) proposed a method for the determination of the reduced scattering coefficient only, using an optical beam of oblique incidence. Moreover, their analysis does not include the effect of the phase function. Kessler et al. (Patents 5,284,137 and 5,645,061) proposed a method for measuring the local dye concentration and scattering parameters in animal and human tissues, based on spatial and spectral measurements. However, their methods do not enable the simultaneous determination of the absorption coefficient, reduced scattering coefficient. Other publications may be of concern:

1. See "Welch, A. J.; van Gemert, M. J. C. Optical Thermal Response of Laser Irradiated Tissue; Plenum publishing Corp., New York, 1995", and references therein.
2. W.-F. Cheong, S.A. Prahl, and A.J. Welch, "A Review of the Optical Properties of Biological Tissues," IEEE J. Quantum Electron. 26, 2166-2185 (1990).

3. Summary of the Invention:

In the case of source-detector separations close to one transport mean free path, light propagation has been modeled using Monte Carlo simulations. In contrast to the previous case where the source-detector separation is larger than several transport mean free paths, it was discovered by the inventors that not only the first moment of the phase function must be considered, but also the second moment. More precisely, the relevant optical properties are the refractive index, the absorption, the reduced scattering coefficient and a new parameter $\gamma = (1-g_2)/(1-g_1)$, that takes into account the first two moments of the phase function, denoted g_1 and g_2 respectively.

Different methods have been proposed for local characterization of turbid media, and in particular of biological tissues. Nevertheless none allows for the simultaneous determination of the absorption, the reduced scattering coefficient and the parameter γ .

In the present invention we present a method and apparatus for the measurement of the absorption coefficient, the reduced scattering coefficient and the said phase function parameter γ , from the spatially-resolved reflectance data at short source detector separation. These three parameters, which can be measured at different wavelengths, enable us to characterize turbid media, such as biological tissues. Our method is based on a theoretical study of the reflectance at short source-detector separations, that we performed with Monte Carlo simulations.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1. Description of the principle of spatially-resolved reflectance measurement.

ρ denotes the source – detector distance, ω_{source} and ω_{detector} are the solid angles of light acceptance determined by the Numerical Apertures (N.A.) of the optical fibers

Fig.2 Probability density function of the mean depth of scattering events per detected photons.

Fig.3. Examples of spatially resolved reflectance curves obtained with different phase functions. Case of matched refractive index ($n=1.0$).

Fig.4. Monte Carlo simulations and fits of the spatially resolved reflectance curves by mathematical expression in the form $R(\rho) \equiv [A(\rho, \mu_s, \gamma) + \mu_s B(\mu_s)]^2$. Case of constant $\gamma=1.9$ and $\mu_s = 1 \text{ mm}^{-1}$, numerical aperture of the source and detectors = 0.37. $A = 0.0647 \rho^{0.324} \exp(-0.161\rho)$, $B = 0.18653 \mu_a - 0.8466 \mu_a^2 + 1.836 \mu_a^3$

Fig.5.a. Basic description of the apparatus: Case of measurements with an optical fiber probe. The apparatus comprises a probe connected to a light source unit and a detection unit, both units being connected to a computer or signal processing unit. The optical fiber probe is constituted of one or more illuminating fibers, and one or more collecting fibers.

Fig.5.b. Basic description of the apparatus: Particular embodiment where the probe is composed of n detecting fibers (typically 6) in parallel. A broadband or white light source is used for excitation and the retro-diffused light is collected by the n detecting fibers in parallel and dispersed in parallel in a spectrograph, before being detected on a 2D CCD camera and transmitted numerically to a PC for γ, μ_s', μ_a determination at each wavelength λ .

Fig.5.c. Basic description of the apparatus. Case of a measurement with sources and detectors directly in contact with the turbid medium.

Fig.5.d. Basic description of the apparatus. Case of non-contact measurements with a 1D or 2D detector, coupled with an imaging device. Scanning systems 1 and 2 are optional, they may be distinct from one another or confounded in a single scanner.

Fig.5.e. Basic description of the apparatus. Case of non-contact measurements with source and detection beams deflected by one or two scanning devices which may be distinct from one another or confounded.

Fig.6. Examples of the sample side of the fiber optical probe.

6a. Simple arrangement for a single measurement.

6b. Arrangement for symmetrical measurements.

6c. Arrangement for multiple measurements.

6d. Arrangement for multiple measurements, using an multicore fiber, or an optical fiber bundle.

6e. Arrangement for symmetrical measurements where detecting fibers are arranged on an elliptic path so as giving a regular spacing from the illuminating fibers to the detecting fibers, while maximizing the collecting surface of the fibers. Additional fibers F can be put in the center of the probe or around the probe for other light channels: collecting fibers for fluorescence or Raman scattering for example.

Fig.7. Example of a measurement of the spatially-resolved reflectance with the probe shown in Fig.6.a. The measurement, performed on a microsphere suspension, is superimposed to a Monte Carlo simulation. The optical properties, computed from published water optical properties (μ_a) and Mie theory (μ_s' , γ) are: $n=1.33$, $\mu_a=0.0004 \text{ mm}^{-1}$, $\mu_s'=1.0 \text{ mm}^{-1}$, $\gamma=2.2$. The calibration was performed with a siloxane sample of known optical properties.

Fig.8. Relation between the parameters $R(\rho=1 \text{ mm})$ and $|\partial \rho \ln R(\rho=1 \text{ mm})|$ and the optical coefficients μ_s' and μ_a . Case of $\gamma=1.5$ and 1.9 . Probe of refractive index = 1.5, sample of refractive index = 1.4, optical fibers diameter = 200 μm , NA=0.37 (source and collection).

Fig.9. Plot of the parameter $\frac{\partial}{\partial \rho} \sqrt{R}$ for different γ values (1.0, 1.8, 2.5), different reduced albedo a' (1, 0.95, 0.9) and for fixed $\rho=1$ mm. Mismatched refractive index $n=1.4$.

Fig.10. Plot of $\sqrt{R(a'=1)} - \sqrt{R(a')}$ for different γ values and reduced albedo a' , and for fixed $\rho=1$ mm. Mismatched refractive index $n=1.4$.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

The present definitions are given for clarity of the concepts developed in the patent and are worded in agreement with ref.1.

The concept of spatially resolved reflectance is illustrated in Fig.1. Consider a collimated light beam impinging on a turbid medium (through air or through a light guide, or even generated locally), in a given solid angle ω_{source} determined by the Numerical Aperture of the illuminating fiber: N.A. which is defined as the half angle of the entrance angle in the fiber, and on a limited area A_{source} , where the source irradiate the surface. The spatially resolved reflectance $R(\rho)$ is defined by the backscattered light power in a given solid angle $\omega_{detector}$, at a distance ρ from the source, per unit area and normalized by the source power. The collecting area of the detector or detecting fiber is $A_{detector}$. The distance ρ is

referred as the source-detector separation (also in the case when light guides or imaging devices are used for the illumination and for the collection of the backscattered light).

$R(\rho)$ depends on the optical properties of the turbid medium, defined below. Note that $R(\rho)$ depends also on the source and detectors characteristics, i.e. numerical aperture and sizes. In a general situation, $R(\rho)$ is the result of the convolution of the corrected reflectance curve and the source irradiance and detector sensitivity over, respectively, the surfaces A_{source} and $A_{detector}$. When A_{source} and $A_{detector}$ are smaller than the required spatial resolution, $R(\rho)$ is confounded with the corrected spatially resolved reflectance.

It is commonly admitted that the fundamental optical properties of a turbid medium is determined by the average index of refraction n of the medium, the absorption coefficient μ_a , the scattering coefficient μ_s , and the phase function $p(\theta)$ where θ is the scattering angle. The absorption coefficient μ_a [mm^{-1}] is defined as the probability of absorption per unit infinitesimal pathlength. The scattering coefficient μ_s [mm^{-1}] is the scattering probability per unit infinitesimal pathlength. The phase function $p(\theta)$ is the density probability function for the scattering angle θ . The phase function is normalized as follows:

$$1 = 2\pi \int_0^\pi p(\theta) \sin \theta d\theta \quad (1.1)$$

In turbid media and in biological tissues in particular, the phase function $p(\theta)$ is a continuous function of the scattering angle θ and generally depends on the dielectric properties and on the material microstructure. It can be approximated by a development in Legendre polynomials:

$$p(\theta) = \frac{1}{4\pi} \sum_{n=0}^{\infty} (2n+1) g_n P_n(\cos \theta) \quad (1.2)$$

Where g_n is the n^{th} order moment of the phase function and $P_n(\theta)$ is the Legendre polynomial of order n :

$$g_n = 2\pi \int_0^\pi P_n(\theta) p(\theta) \sin \theta d\theta \quad (1.3)$$

The first moment of the phase function is also called the anisotropy factor, and is often simply noted g ($=g_1$). It represents the mean cosine of the scattering angle. The reduced scattering coefficient μ_s' is defined as:

$$\mu_s' = \mu_s(1-g) \quad (1.4)$$

The transport mean free path (or reduced mean free path) mfpp' of the photons, in a turbid medium, is defined as:

$$\text{mfpp}' = (\mu_s' + \mu_a)^{-1} \quad (1.5)$$

The reduced albedo a' is the ratio:

$$a' = \mu_s' / (\mu_s' + \mu_a) \quad (1.6)$$

As a result of the present invention described in more details in the next section, it is also necessary to define another phase function parameter called γ :

$$\gamma = (1-g)/(1-g_1) \quad (1.7)$$

All the parameters listed above are referred as optical properties. They are wavelength dependent, and can vary in space and time. In the following, μ , g_1 and g_2 will be considered as parameters characterizing the turbid medium.

2. Reflectance measurements at distances close to one transport mean free path

The methods described in the US Patent 5,517,987 (Tsuchiya) are based on measurements with large range of source-detector separations, typically from 1 mfp' to 10 mfp'. In such cases, the volume probed is on the order of $10-1000 (\text{mfp}')^3$. In contrast with such a large scale investigation, the present invention is directed to a novel approach where the volume probed is much smaller, on the order of $1 (\text{mfp}')^3$. This is achieved by using only small source detector separations, typically from 0.1 to 2 mfp'. The lateral dimension of probing is limited to this range of distances.

Note that the present invention can be complemented by large source-detector separation measurements, if the optical properties of both superficial and deep parts of a turbid medium must be determined.

A model of photon migration in tissue was necessary to predict the relationship between the measured reflectance and the optical properties. Analytical solutions from the diffusion equation are not appropriate in our case because we are interested in the reflectance close to the source, at distances comparable to the transport mean free path [mfp']. We have performed Monte Carlo simulations to predict the measured reflectance of an homogeneous semi-infinite turbid media.

The exact diameter of the illuminating and collecting fibers, as well as their Numerical Apertures (N.A.), have been taken into account in the simulations. The mismatch of index of refraction at the surface of the medium have been also taken into account in the simulations, by using the Fresnel law for each photon reaching the surface.

This is also one result of this simulation to compute the average depth of probing, illustrated in Fig.2. . It is demonstrated that only the superficial part of the turbid medium is probed if small source-detector separations are used. For this, we determined the depth below the surface of each scattering event in the simulation. With this information we determined the average depth of all the scattering events for each detected photon, which we present as a probability density function in Fig.2. . This figure shows that the average depth of scattering is approximately around 1 mfp'. Moreover it showed that the part located below 2 mfp' were not playing a significant role in the measured signal (for $\rho < 1.5$ mfp').

The spatially resolved reflectance $R(\rho)$, with short source-detector separations is more complex than in the case of large source-detector separations. Indeed, for small source detector-separation conditions, the inverse problem. i.e. calculating the localized absorption and reduced scattering coefficients, is sensitive to the scattering phase function. It is part of the present invention to have shown, from Monte Carlo simulations, that only the first and second moments of the phase function can be taken into account. Moreover, it was established that the influence of these two moments are not

independent. Indeed, it is a merit of this invention to demonstrate that only one parameter $\gamma = (1-g_2)/(1-g_1)$, which depends on the first and second moments of the phase function can be used to characterize accurately the reflectance at short source-detector separation.

Fig.3 illustrates the fact that the parameter γ is the only predominant parameter of the phase function that must be taken into account (and not $g=g_1$ as frequently mentioned in literature). Different reflectance curves, obtained from Monte Carlo simulations are shown in Fig.3. Four different phase functions were used for the simulations. Three phase functions are characterized by $\gamma=1.25$, but different g_1 and g_2 values. Fig.3. shows that almost identical reflectance curve are obtained for distances $\rho\mu_s' > 0.3$, if the g_1 and g_2 are varied in such a way that the parameter γ stays constant ($\gamma=1.25$). For comparison, the reflectance computed with a different g ($=g_1$) value is also presented ($\gamma=2.25$). It appears then clearly that the parameter g ($=g_1$) plays a significant role in the reflectance.

The parameter γ depends on the characteristics of the set of scatterers inside the turbid medium: shape, distribution of sizes and refractive index.

The possibility of determining this parameter γ is an important part of this invention, and has never been reported earlier. The determination of γ can be related to the microscopic structure of the sample, since γ is related to the sizes and refractive indexes of the scatterers.

In most cases, the average refractive indices n and the source and detectors characteristics are fixed and known. In such case, only three parameters are determined from measurements at short source-detector separation: μ_a , μ_s' and γ .

It is important to note that the parameter γ cannot be estimated if large source detector separations are used. Indeed, the reflectance is only sensitive to γ at short source-detector separation.

It was also derived from Monte Carlo simulations that the reflectance $R(\rho)$, for $\rho \approx 1 \text{ mfp}$, can be reasonably well approximated by the expression:

$$R(\rho) \cong [A(\rho, \mu_s', \gamma) + B(\mu_a, \mu_s')]^2 \quad (1.8)$$

In many cases, we found that Equ.(1.8) can also be written as:

$$R(\rho) \cong [A(\rho, \mu_s', \gamma) + \mu_s' B(\mu_a)]^2 \quad (1.9)$$

it is important for the description of the invention to evidence the fact that the function A depends on ρ , the scattering properties (i.e. μ_s' and γ) but not on μ_a . In contrast, the function B depends on μ_a and μ_s' but neither on γ nor ρ . An example of Equ. (1.9) is shown in Fig. 4.

This formulation is of great help to solve the inverse problem, as described in section 5.3.

3. Apparatus

In the first embodiment, The apparatus can be divided in three parts, described in Fig.5.a.

The first part is the illuminating system. Any light source can be used. For examples: a) white sources, such as halogen or xenon lights, metal halides or fluorescent or phosphorescent sources. b) sources such as lasers, laser diodes, optical fiber lasers, light emitting diodes or superluminescent diodes. c) the sources described in point a) and b) where monochromators, filters or interference filters are added to select a given set of wavelengths.

In the first preferred embodiment, The light power is conducted to the investigated sample by the probe, which is the second part of the apparatus. The probe is preferably made of optical fibers, to illuminate and to collect the backscattered light. But GRIN rods or other type of light pipes can also be used. Different possible arrangements of optical fibers are illustrated in Fig.6 . Two different modes of measurements can be chosen. First, one fiber is used to illuminate the sample and at least two other fibers are used to collect the backscattered light at two different distances. Second, one fiber is used to collect the light and at least two other fibers, located at two different distances from the first one, are used to illuminate sequentially the sample.

The arrangement of the different fibers can be replaced by any imaging system or image guide, such as multicore optical fibers, or optical fiber bundle.

The light collected by the probe is analyzed by the detection unit, which is the third part of the apparatus. If wide spectral light sources are used (such as halogen or xenon lights), a spectrograph can be put between the probe and the detector to get wavelength dependence of the backscattered signal (either in the source or detection unit). Any types of detectors can be used. For example, photodiodes, avalanche photodiodes or photomultipliers can be assigned to each collecting fibers. Simultaneous detection of each collecting fiber can also be achieved using linear or two-dimensional detectors such as Charge-Coupled Detectors (1D or 2D), intensified CCD or array of photodiodes.

A particular embodiment is described in Fig.5.b. : the probe is composed of n detecting fibers (typically 6). A broadband or white light source is used for excitation and the retro-diffused light is collected by the n detecting fibers and dispersed in parallel in a spectrograph, before being detected by a 2D CCD camera and transmitted numerically to a PC for γ , μ_s , μ_a determination at each wavelength λ . This particular embodiment has the advantage of yielding the wavelength dependence of γ , μ_s , μ_a from a single measurement.

A second type of embodiment is presented on Fig. 5.c. The difference with embodiments presented in Fig. 5.a. and Fig. 5.b. is that optionally no optical fibers, light pipes or grin rods is used. The light source unit is directly in contact with the turbid medium, as well as the detector unit. Collimating optics, micro-optics or imaging optics (DOE Diffractive Optical Elements for example) can be put between the turbid medium and the actual light

sources and detectors. The different type of sources and detectors cited in example for the first embodiment can be used for the second type of embodiments. Hybrid design, such as arrangements involving both direct contacts sensors or detectors and fibers, light pipes or grin rods are also included in the present embodiment.

The third embodiment is described in Fig.5.d. and Fig.5.e. The only difference with the first and second embodiment is that non-contact measurements are performed. A collimating system allows for a point-like illumination on the turbid medium. An imaging system enables the measurement of the spatial distribution of the reflectance. The detectors can be either an array (1D or 2D) of detectors (Fig.5.d) with optional scanning systems 1 and 2 which can operate separately or can be confounded in a single scanner, or a single detector (Fig.5.e). In this last case, a scanning device is used to obtain the spatially-resolved reflectance. A fiber bundle, multicore fiber or relay optics (grin rod or multiple lenses) can be put between the focal point of the imaging system and the detector(s). The different type of sources and detectors cited in example for the first embodiment can be used for the third embodiment.

Spatial images of the parameters μ_a , μ_s' and γ can be obtained by a series of measurements at different locations, that we call multi-site measurements. Some mechanical or optical scanning device can be used for this purpose. The resolution of such images is on the order of the mean source-detector separation used for a single site measurement. All three embodiments can be expanded to perform multi-site measurements, by duplicating and/or multiplexing the illuminating or measuring devices.

For example, the optical fiber probes shown in Fig.6., can be duplicated and put side by side. The scanning system described in the third embodiment can also be expanded to perform multi-site measurements.

4. Normalization and calibration

The differences of transmission between each fiber are corrected by performing a measurement on a turbid phantom illuminated uniformly.

The background light, measured with the light source turned off, must be subtracted from the signal.

In order to perform absolute intensity measurements, a calibration is performed on a turbid medium of known optical properties. Examples of such a medium are: 1) solid or liquid turbid medium which properties have been measured by other techniques, or reported in the literature, 2) water suspension of microsphere of known size distribution and refractive index. Absorbing dye may be added to the suspension. In case 2) the scattering properties are calculated from Mie theory, and the absorption coefficient is assumed to be equal to the water absorption coefficient, if no absorbing dye is added. If an absorbing dye is used, the absorption coefficient can be measured by a spectrophotometer, before mixing the solution with any scattering materials.

A Monte Carlo simulation is performed with the optical properties of the calibration sample. The simulation is then divided by the experimental reflectance performed on the

calibration sample. The result, that must be independent of the source-detector separation, is defined as the calibration factor. Each new measurement is multiplied by the calibration factor.

An example of a measurement, after calibration, is shown in Fig.7. The measurement was performed with the apparatus described in the first embodiment, with a probe similar to the one described in Fig.6.a. A prior calibration was performed on a solid turbid medium, which optical properties were measured by another technique (frequency domain measurement, cf. Ref 1.). The sample was a water suspension of polystyrene microsphere of 1 μm diameter. The measurement is compared to a Monte Carlo simulation performed with the scattering properties calculated from Mie theory and absorption coefficient of water. shows an excellent agreement between the measurement and the simulation, which confirm the validity of our simulation model, experimental measurement and calibration.

5. Signal processing

5.1. Control of the homogeneity of the area probed

Artifacts during a measurement, for example due to bad contact between the probe and the sample, or heterogeneity of the sample, can be detected by the following optional procedure. Two illuminating fibers are disposed symmetrically in regard to the collecting fibers (see Fig.6.b). If the sample is homogeneous, the reflectance curve should be identical with either illuminating fiber. Therefore, heterogeneity of the investigated region or obstructions beneath the fibers are detected by comparing the two curves. If the

two curves are sufficiently close, the measurement is validated and the average of the two curves is calculated.

5.2. Smoothing procedure and computation of the derivative of the curve.

Functions in the form $m_1 \rho^{m_2} \exp(m_3 \rho)$ were always found to fit well Monte Carlo simulations for a restricted range of distances. Therefore, a smoothing of the experimental reflectance $R(\rho)$ can be processed by fitting $R(\rho)$ to $m_1 \rho^{m_2} \exp(m_3 \rho)$.

The determination of the slope of the logarithm $\frac{\partial}{\partial \rho} (\ln R(\rho, \mu_s, \mu_a, \gamma))$ is also derived from this fit, using the following formula:

$$\frac{\partial}{\partial \rho} (\ln R(\rho, \mu_s, \mu_a, \gamma)) = \frac{m_2}{\rho} + m_3 \quad (1.10)$$

The slope of the square root of $R(\rho)$ can also be derived (the use of these slopes are described below in section 5.3):

$$\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)} = \left(\frac{m_2}{\rho} + m_3 \right) \left(\sqrt{m_1} \rho^{\frac{m_2}{2}} \exp\left(\frac{m_3 \rho}{2}\right) \right) \quad (1.11)$$

5.3. Inverse problem

The so-called "direct problem" consists in computing the spatially resolved reflectance $R(\rho)$ from the values of the optical coefficients n, μ_a, μ_s, γ involved in a model of

propagation of the light in turbid medium and whereby the said "model" incorporates a Legendre polynomial development to the second order of the said "phase function", and whereby the said "phase parameter" γ is introduced in the computation as an independent parameter. The so-called "inverse problem" consists in extracting the optical coefficients n, μ_a, μ_s', γ from the spatially resolved reflectance data $R(\rho)$.

We developed different methods to solve the inverse problem, which consists in extracting optical coefficients from the reflectance data.

Method 1.

Monte Carlo simulations show that the measurements of the reflectance intensity $R(\rho)$ and the slope of $\ln R(\rho)$ (denoted $\partial \rho \ln R$), determined at a fixed distance ρ , can be used to derive μ_s' and μ_a for a given γ value. Fig.8. shows graphically the relationship between μ_s' and μ_a and the two parameters $R(\rho=1 \text{ mm})$ and $|\partial \rho \ln R(\rho=1 \text{ mm})|$ in the case of $\rho=1 \text{ mm}$, $\gamma=1.5$ and 1.9 . Note that any other choice of ρ is possible, as long as ρ is on the order of $1 \text{ mfp}'$. We see in Fig.8. that μ_s' and μ_a can not be determined uniquely if γ is unknown. Further insight for optimizing the inversion strategy is provided by three additional features of Fig.8.

First, the determination of μ_s' is only weakly influenced by γ . Indeed in Fig.8. the errors induced by error in γ are typically 10% for μ_s' around 1 mm^{-1} . Second, although absolute determination of μ_a is not possible when γ is not precisely known, relative absorption changes can be still evaluated. Third, the indetermination of the parameter γ may be

resolved by the values of $R(\rho)$ and/or $|\partial \rho \ln R(\rho)|$ at other distances. Therefore the following procedure can be used:

- (1) determination of μ_s' and μ_a from $R(\rho=1 \text{ mm})$ and $|\partial \rho \ln R(\rho=1 \text{ mm})|$ for a set of values γ . For example: $\gamma=1.0, 1.1, 1.2, \dots, 2.5$.
- (2) simulations with the different sets of μ_s' and μ_a obtained from point 1)
- (3) comparison between the simulations and the reflectance profile for distances $0.3 < \rho < 2 \text{ mm}$.

This last step allows us to determine the correct values of γ , μ_s' and μ_a . Points 1 to 3 can be done iteratively to evaluate γ more precisely, using a finer discrimination of γ values.

This method described by the iteration of points 1) to 3) corresponds to finding a simulation that fit closely a measured curve $R(\rho)$. This can also be achieved by directly comparing the measured curve $R(\rho)$, with a set of simulations, and finding the simulation that minimize the square of the differences between the measured and simulated curves.

Note also that the use of the reflectance intensity $R(\rho)$ and the slope of $\ln R(\rho)$ is equivalent to the use of two intensities $R(\rho_1)$ and $R(\rho_2)$, measured at two close distances ρ_1 and ρ_2 , respectively.

Method 2.

The inverse problem can also be performed, considering properties of $R(\rho)$ expressed by Equ.(1.8).

Indeed, it can be derived from Equ.(1.9) that the quantity does not depend on the absorption coefficient μ_a :

$$\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)} = \frac{\partial A}{\partial \rho}(\rho, \mu_s, \gamma) \quad (1.12)$$

This property is confirmed in Fig.9 , where the quantity $\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)}$, evaluated at $\rho=1$ mm, is plotted as a function of μ_s' for $\gamma=1, 1.9$ and 2.5 and reduced albedo $a'=1, 0.95$ and 0.9 (note that any other choice of ρ is possible, as long as ρ is of the order of 1 mfp').

In Fig.9. , the parameter $\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)}$ clearly depends on μ_s' and γ . In contrast, the dependence on a' is almost negligible.

Therefore, γ and μ_s' can be derived from the parameter $\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)}$, calculated from the experimental reflectance $R(\rho)$ (see section 5.2). Simultaneous determination of γ and μ_s' require values of $\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)}$ at different distances ρ (at least two). If one of the parameters γ or μ_s' is already known, the determination of the other one can be obtained from the value of $\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)}$ at a single distance.

Convenient analytical approximation of $\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)}$ can be obtained by fitting Monte Carlo results to polynomial functions. For example, in the case of fixed $\gamma=1.9$, we obtained:

$$\mu_s' = 0.9162 - 52.89x + 1795x^2 - 18155x^3 + 65428x^4 \quad (1.13)$$

where $x = \frac{1}{2} \frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)}$ at $\rho=1$ mm. x is expressed in $[\text{mm}^{-2}]$ and μ_s' is expressed in $[\text{mm}^{-1}]$.

This results is valid for a probe of refractive index of 1.5 and a sample of refractive index of 1.4, optical fibers of diameter 200 μm , NA=0.37 (source and collection).

Once μ_s' and γ are calculated from the procedure explained above, μ_a is calculated from the absolute value of $R(\rho)$, which highly depends on μ_a . For given μ_s' , γ and ρ values, the dependence of reflectance on μ_a is obtained by Monte Carlo results. From Equ.(1.8) we have:

$$\mu_a = h \left[\sqrt{R(\rho, \mu_s, \mu_a, \gamma)} - f(\gamma, \mu_s') \right] \quad (1.14)$$

where h and f are functions given by Monte Carlo simulation. Particularly, they can be well approximated by polynomial functions. For example, for $\gamma=1.9$, probe refractive index of 1.5, sample refractive index of 1.4, optical fibers of diameter 200 μm , NA=0.37 (source and collection):

$$h = -0.002257 - 8.171 \gamma + 268.8 \gamma^2 \quad (1.15)$$

$$f = 0.01311 + 0.05184 \mu_s' - 0.01974 \mu_s'^2 + 0.003217 \mu_s'^3 - 0.0001992 \mu_s'^4 \quad (1.16)$$

where $\gamma = \left[\sqrt{R(\rho, \mu_s', \mu_a, \gamma)} - f \right] [\text{mm}^{-1}]$. μ_a is expressed in $[\text{mm}^{-1}]$.

Method 3

Equ.(1,8) also shows that relative measurements of μ_a , i.e. variation of μ_a from a known value μ_{a0} , is possible by measuring the variation of the parameter $\sqrt{R(\rho, \mu_s', \mu_a, \gamma)}$:

$$\sqrt{R(\rho, \mu_s', \mu_{a0}, \gamma)} - \sqrt{R(\rho, \mu_s', \mu_a, \gamma)} = B(\mu_{a0}, \mu_s') - B(\mu_a, \mu_s') \quad (1.17)$$

This relation is illustrated in Fig.10. in the case of $\mu_{a0}=0$, $\rho=1 \text{ mm}$, $\gamma=1.0, 1.9$ and 2.5 , and for $a' = 1$ to 0.83 . confirms that the influence of γ is weak in the quantity $\sqrt{R(\rho, \mu_s', \mu_{a0}, \gamma)} - \sqrt{R(\rho, \mu_s', \mu_a, \gamma)}$. For known μ_s' , the function $B(a')$ allows for the determination of a relative absorption change $\Delta\mu_a = \mu_a - \mu_{a0}$. Fig.10. illustrates the case $\mu_{a0}=0$, but any other value of μ_{a0} is possible. The interesting point is that $B(a')$ does not depend on the phase function.

CLAIMS

1. A method for local and superficial (probed volume and measuring area with dimensions on the order of one transport mean free path) characterization of a turbid medium using the following parameters:

- 1) the refractive index n of the turbid medium
- 2) the absorption coefficient μ_a of the turbid medium,
- 3) the reduced scattering coefficient μ_s' of the turbid medium,
- 4) the phase function parameter $\gamma = (1-g_2)/(1-g_1)$ of the turbid media, where g_1 and g_2 are the first two moments of the Legendre polynomial development of the phase function p ,

and comprising the steps of:

- measuring the spatially-resolved reflectance $R(\rho)$ of the turbid medium (ρ being the source-detector distance) by any mean, comprising an illumination beam as a source and an optical detector, which, by using optional signal processing, which may involve filtering and de-convolution operation to correct for the non-zero area of either the illumination source or the detector, allows for the precise determination of the said spatially-resolved reflectance $R(\rho)$,
- mathematically processing $R(\rho)$ to compute at least one of the said parameters: n , μ_a , μ_s' , γ and/or the variations, in time and/or space, of at least one of the said parameters: Δn , $\Delta \mu_a$, $\Delta \mu_s'$, $\Delta \gamma$, whereby the said "inverse problem", which consists in extracting the optical coefficients from the spatially resolved

reflectance data is solved, and whereby the said "direct problem" consists in computing the spatially resolved reflectance from the values of the optical coefficients n , μ_a , μ_s , γ involved in a model of propagation of the light in turbid medium and whereby the said "model" incorporates a Legendre polynomial development to the second order of the said "phase function", and whereby the said "phase parameter" γ is introduced in the computation as an independent parameter.

2. The method of claim 1, wherein said spatially resolved reflectance is measured by a probe comprising at least one optical fiber carrying the light from the source unit to the turbid medium and at least one optical fiber collecting the reflected light and carrying it to detection unit, whereby the combination of a variety of emitting fibers and of receiving fibers yields a set of distances ρ at which the reflectance $R(\rho)$ is measured.
3. The method according to any one of claims 1 and 2, wherein said spatially resolved reflectance $R(\rho)$ is measured for a set of values of ρ , by using a probe composed of optical fibers in any of the following configurations:
 - one emitting optical fiber and a set of optical receiving fibers
 - a set of optical emitting fibers and one optical receiving fiber
 - a set of optical emitting fibers and a set of optical receiving fibers

giving the spatially resolved reflectance $R(\rho)$ at a variety of source - detector distance ρ . The emitting and receiving fibers can be arranged in a variety of configurations. In some particular arrangement they can be positioned:

- on a line,
- on crossed lines,
- on a circle,
- on an ellipse
- on crossed ellipses
- on a disk, a rectangle, or any surface, as a dense and contiguous arrangement of fibers, such as it can be obtained with a bundle of fibers or a multi-core fiber,
- on any pattern resulting from the combination of the above mentioned patterns.

4. The method according to any one of claims 1, 2 and 3, wherein said spatially resolved reflectance is measured by an optical and electronic micro-system comprising a collimated or focused beam as illuminating source and 1D or 2D arrays of optical detectors, arranged in a way similar to the one described in claim 3, and which, in some embodiment, can be a CCD or MOS camera.
5. The method according to any one of claims 1-4, where the probe of claims 2 and 3 or the optical and electronic micro-system of claim 4 are put in contact to the turbid medium.

6. The method of claim 1, wherein said spatially resolved reflectance is measured by a non-contact system, comprising at least one of the following combination of optical systems:

- a fixed optical system to irradiate the turbid medium with a collimated or focused beam forming the illuminating source and a fixed optical system comprising an imaging system forming the image of the measured area of the turbid medium on a said "optical detector", which can be formed of 1D or 2D array of optical detectors, whereby this second optical system can be identical to the first one and whereby the array of optical detectors can be either one of the following system:

- a set of optical fibers arranged in a variety of configurations similar to those described in claim 3,
- an optical and electronic micro-system (MOEM), which in some embodiment can be a CCD or MOS camera, with a functionality similar to the one described in claim 4,

- a fixed optical system for the collimated beam illuminating source and a scanned optical system for the said "optical detector",

- a scanning optical system for the collimated beam illuminating source and a fixed optical system for the said "optical detector",

- a scanning optical system for the collimated or focused beam used as an illuminating source and a scanning optical system for the said "optical detector".

7. The method according to any one of claims 1. to 6., wherein the absorption coefficient μ_a , the reduced scattering coefficient μ_s' and the phase function

parameter γ are determined by fitting the measured spatially-resolved reflectance $R(\rho, \mu_r, \mu_a, \gamma)$ to a set of discretized data obtained by using Monte Carlo simulations, or to interpolating functions giving a continuous approximation of the discretized data obtained by Monte Carlo simulations, and whereby said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order. In a preferred embodiment, cubic splines are used as interpolating functions.

8. The method according to any one of claims 1 to 7, wherein the following signal processing steps can be performed in addition to the processing steps of any of the claims 1-6 whereby the processing of the spatially resolved reflectance data of claim 7 can be simplified and accelerated by:

- fitting the measured reflectance $R(\rho)$ by the function:

$$m_1 \rho^{m_2} \exp(m_3 \rho)$$

This fit gives the values of the parameters m_1 , m_2 and m_3 , assuming that the expression $R(\rho) = m_1 \rho^{m_2} \exp(m_3 \rho)$ gives a smoothed expression of the spatially resolved reflectance $R(\rho)$.

- Computing the slopes $\frac{\partial}{\partial \rho} \sqrt{R(\rho)}$ and $\frac{\partial}{\partial \rho} (\ln R(\rho))$, or any mathematical

combinations of these two latter quantities and $R(\rho)$, from analytical functions using the parameters m_1 , m_2 , m_3 , or by numerical procedures from the expression

$$R(\rho) = m_1 \rho^{m_2} \exp(m_3 \rho)$$

- computing the values of at least one of the said parameters: n , μ_a , μ_s , γ and/or the variations, in time and/or space, of at least one of the said parameters: Δn , $\Delta\mu_a$, $\Delta\mu_s$, $\Delta\gamma$ from the relationship between the reflectance intensity $R(\rho)$ and the slope of $\ln R(\rho)$ (denoted $\partial \rho / \ln R$), determined at a fixed distance ρ comparable to the transport mean free path, whereby the computation is made from the data obtained by Monte Carlo simulations, provided that said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

9. The method according to any one of claims 1 to 7, wherein the following signal processing steps can be performed in addition to the processing steps of any claim 1-6 whereby the processing of the spatially resolved reflectance data of claim 7 can be simplified and accelerated by:

- computing the reduced scattering coefficient μ_s' and the phase function parameter γ by using the following form of the reflectance:

$$R(\rho) = (A(\rho, \gamma, \mu_s') + B(\mu_a, \mu_s'))^2$$

where the function $A(\rho, \gamma, \mu_s')$ and $B(\mu_a, \mu_s')$ depend also on the sources and detectors characteristics, and the refractive index of the sample, and comprising the steps of:

- Computing the slopes of the square root of the spatially resolved reflectance

$$\frac{\partial}{\partial \rho} \sqrt{R(\rho, \mu_s, \mu_a, \gamma)} = \frac{\partial A}{\partial \rho}(\rho, \mu_s, \gamma), \text{ which depends weakly on the absorption}$$

coefficient μ_a for $0.3 < \rho \mu_s' < 5$, for at least two distances ρ ,

- Determining the parameters μ_s' and γ by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

10. The method of claims 9, wherein the absorption coefficient μ_a is determined by using the equation:

$$\mu_a = h \left[\sqrt{R(\rho, \mu_s, \mu_a, \gamma)} - f(\gamma, \mu_s) \right]$$

where f and h are continuous functions of the parameters ρ , μ_s' and γ of that can be approximated by a polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order.

11. The method according to any one of claims 1 to 7, wherein the difference $\Delta \mu_a = \mu_a - \mu_{a0}$ between the absorption coefficient μ_a and a known value μ_{a0} is computed from the quantity $\sqrt{R(\rho, \mu_s, \mu_{a0}, \gamma)} - \sqrt{R(\rho, \mu_s, \mu_a, \gamma)} = B(a') - B(a_0')$, whereby the function $B(a')$ of the albedo a' can be approximated by a

polynomial interpolation of the data obtained by Monte Carlo simulations, whereby said "Monte Carlo simulations" are based on a photon propagation model comprising a phase function approximated by a Legendre polynomial development limited to the second order, and whereby the calculation can be done for a single γ value, because the influence of the phase function parameter and γ in $B(a')$ are particularly weak.

12. The method according to any one of claims 1 to 11, wherein the illuminating source is a broadband source or a white light source and the detector unit comprises a spectrograph or any wavelength analysis system to measure the wavelength dependence of at least one of the parameters $(n, \mu_a, \mu_s, \gamma)$.
13. The method according to any one of claims 1 to 12, wherein said turbid medium is a biological medium.
14. The method according to any one of claims 1 to 13, wherein the measurement and processing is repeated at different location of the sample (multi-site measurements), to build images of any one of the said parameters $(n, \mu_a, \mu_s, \gamma)$.
15. An apparatus using the method of claim 1 to 3, 5 or 7 to 14 for local and superficial characterization of a turbid medium,
 - a) comprising a source, a detection unit, reference means, signal processing means, a probe comprising at least one optical fiber connecting said source unit to the

turbid medium and at least one optical fiber connecting the turbid medium to the said detection unit, and reference means

b) where the distance between the source and the detector is close to one transport mean free path

16. An apparatus using the method of claim 1, 4, 5, or 7 to 14 for local and superficial characterization of a turbid medium,

a) comprising an optical and electronic micro-system comprising at least one illuminating source, at least one detector, signal processing means and reference means,

b) where the distance between the source and the detector is close to one transport mean free path

17. An apparatus using the method of claim 1 or 6 to 14 for local and superficial characterization of a turbid medium,

a) comprising a collimated or focused beam used as an illuminating source, at least an optical detector for the detection unit, a fixed or scanning optical system for the illuminating source and a fixed or scanning optical system for the said "optical detector", signal processing means and reference means,

b) where the distance between the source and the detector is close to one transport mean free path

18. Three apparatus similar to the apparatus described in any one of the claims 15, 16 and 17 , and characterized by the fact that the distance between the collimated or focused optical beam used as illuminating source and the emitting point connected to an optical detector varies from 0.1 to 2mm. for application to biological media and to turbid media having a transport mean free path similar to biological media.
19. A test , where the control of the homogeneity of the sample over the probed area is performed with the apparatus of any one of the claims 15, 16,17 and 18 , which can be carried out according to the following optional procedure: In the apparatus two illuminating fibers are disposed symmetrically in regard to the collecting fibers. If the sample is homogeneous, the reflectance curve should be identical with either illuminating fiber. Therefore, heterogeneity of the investigated region or obstructions beneath the fibers are detected by comparing the two curves. If the two curves are sufficiently close, the measurement is validated and the average of the two curves is calculated.
20. A calibration and normalization procedure, which are carried out optionally with the apparatus of any one of the claims 15, 16,17 and 18 , whereby the following steps are performed:
- 1) In order to perform relative intensity measurements, the differences of transmitted intensity between each fiber for the apparatus of claim 15 or the differences of signal intensity between each optical detector for any of the claims 16 and 17, are determined by performing a measurement on a turbid

phantom illuminated uniformly or a diffusing sphere of perfectly uniform properties. In this calibration procedure, the background light, measured with the light source turned off, must be subtracted from the signal. The obtained values are used to multiply the measurements given by each fiber and/or detector to correct the relative intensity measurements.

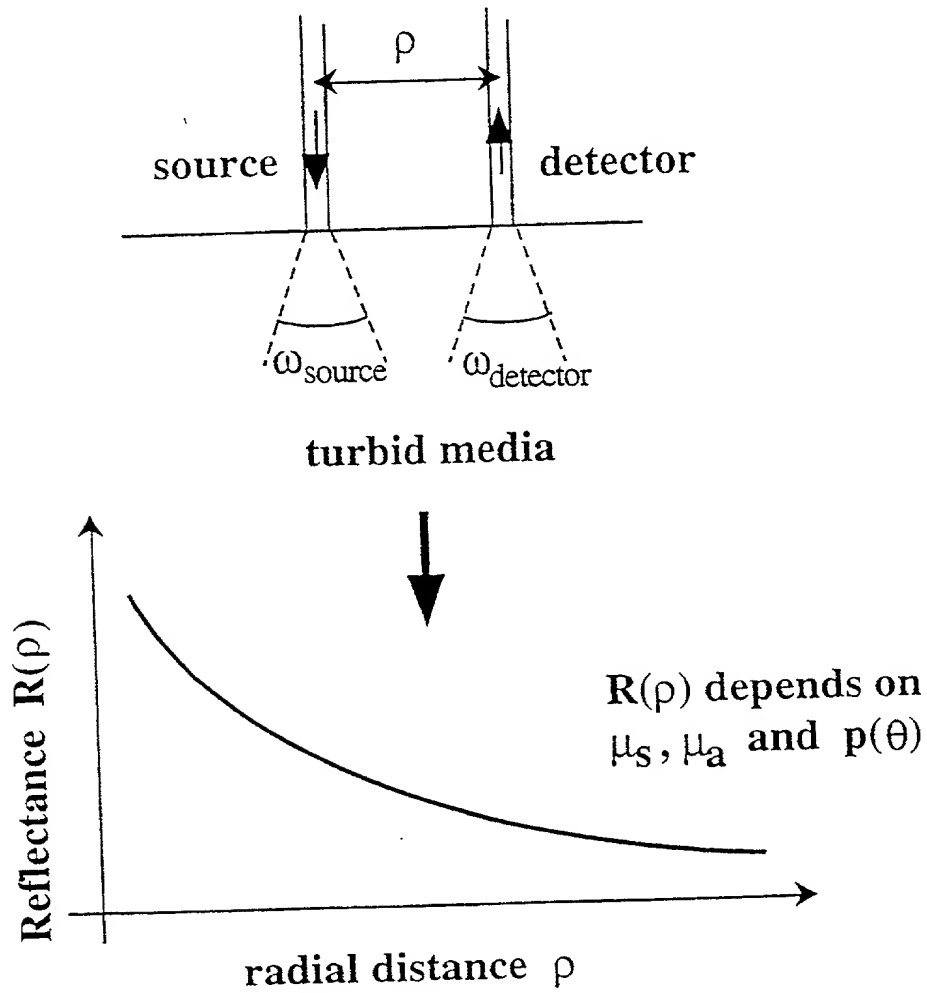
2) In order to perform absolute intensity measurements, a calibration can be performed on a turbid medium of known optical properties, which can be realized according to any one of the following recipe:

- a) solid or liquid turbid medium which properties have been measured by other techniques, or reported in the literature,
- b) water suspension of micro-spheres of known size distribution and refractive index. Absorbing dye may be added to the suspension. In this case, the scattering properties are calculated from Mie theory, and the absorption coefficient is assumed to be equal to the water absorption coefficient, if no absorbing dye is added. If an absorbing dye is used, the absorption coefficient can be measured by a spectrophotometer, before mixing the solution with any scattering materials.

A Monte Carlo simulation is performed with the optical properties of the calibration sample. The simulation is then divided by the experimental reflectance performed on the calibration sample. The result, that must be independent of the source-detector separation, is defined as the calibration factor. Each new measurement can be multiplied by the calibration factor.

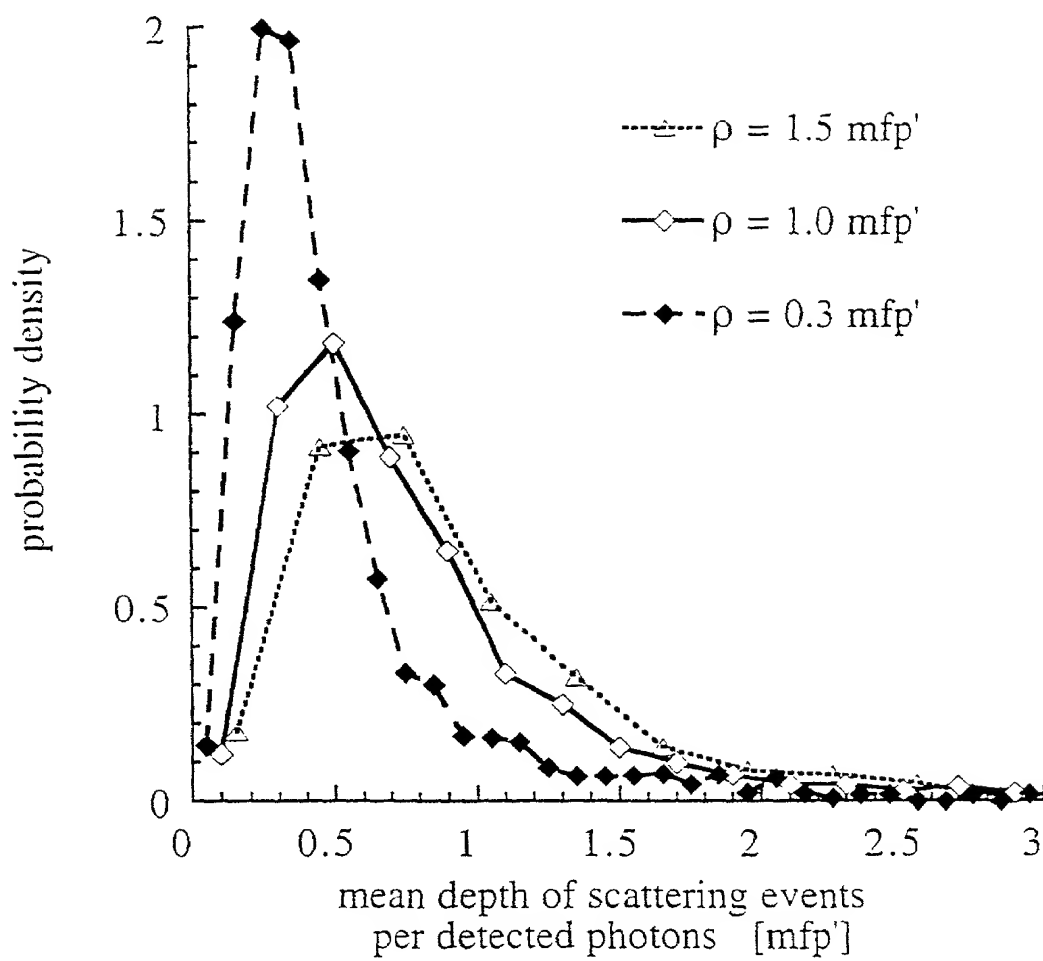
01/15

Fig.1



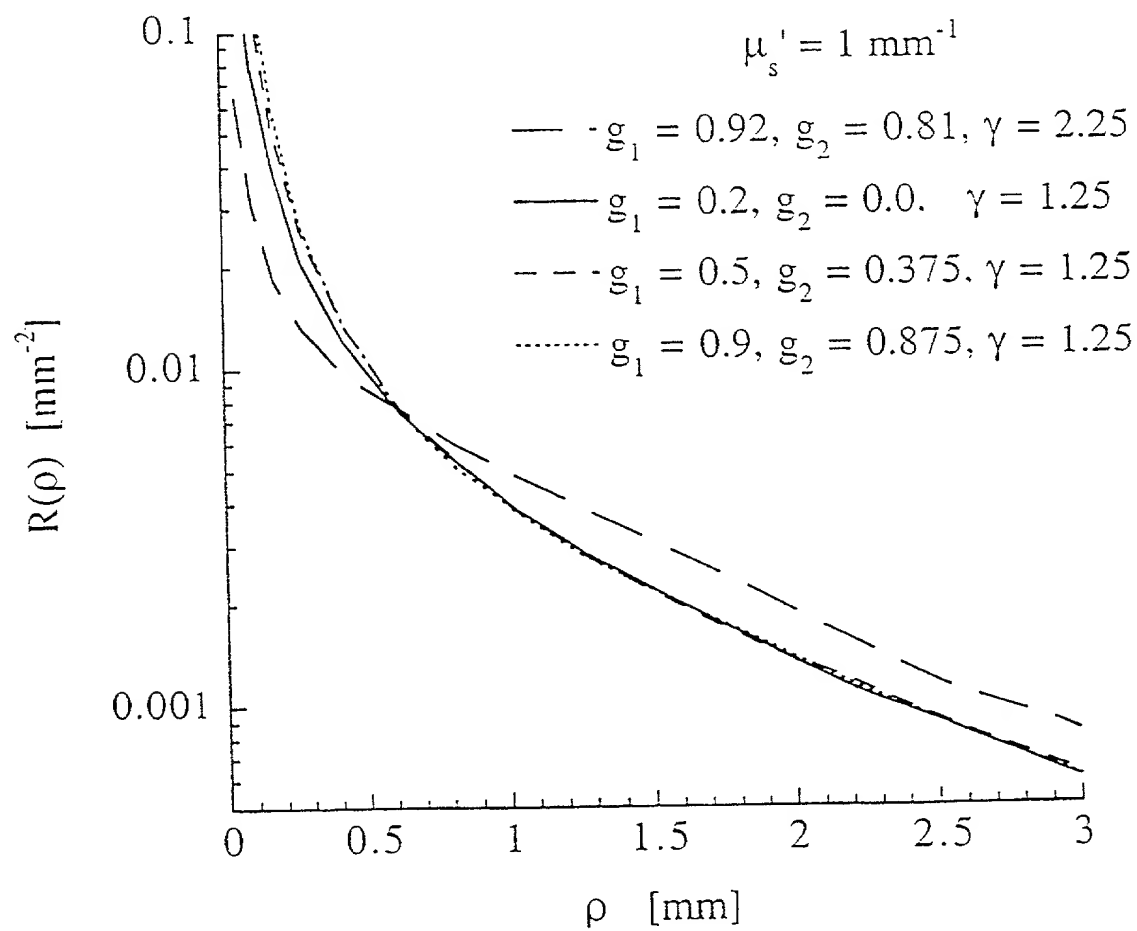
02/15

Fig.2



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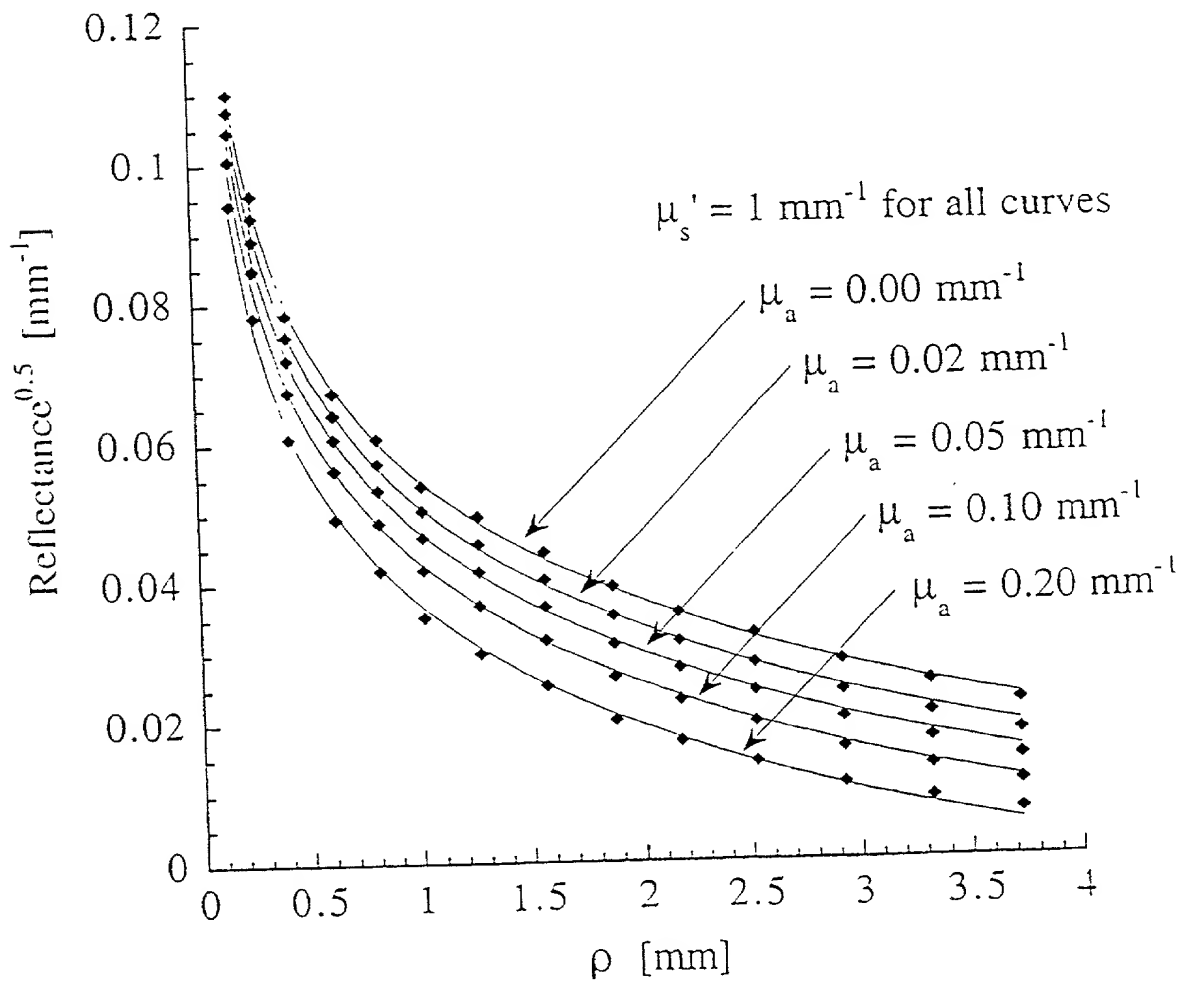
Fig. 3



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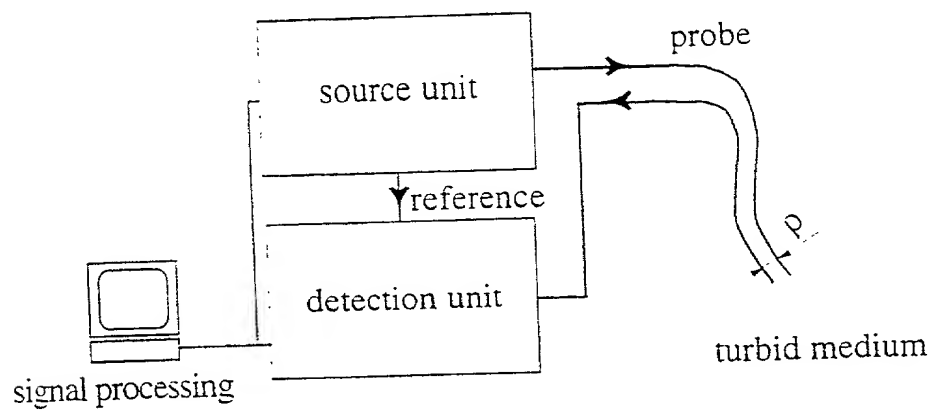
Fig. 4

◆ Monte Carlo simulations

— analytical form $R^{0.5} = A(\rho, \mu_s', \gamma) + \mu_s' B(\mu_a)$ 

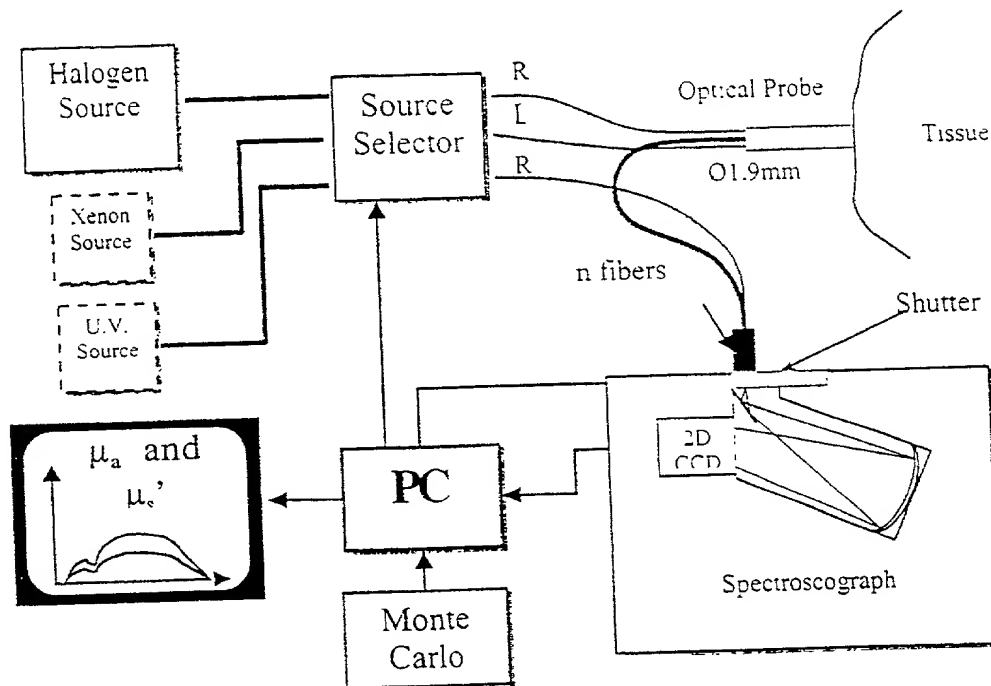
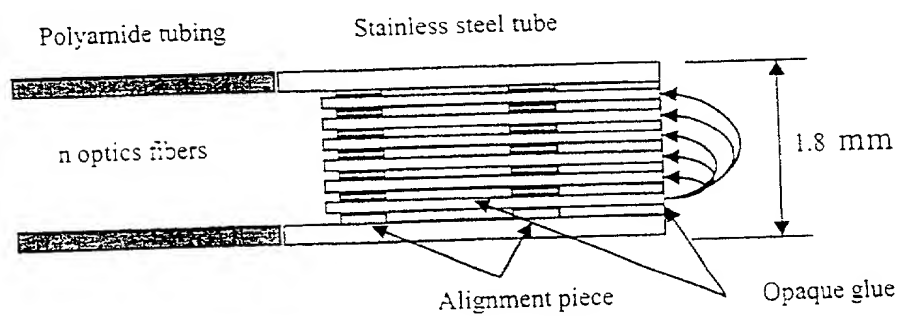
05/15

Fig. 5a



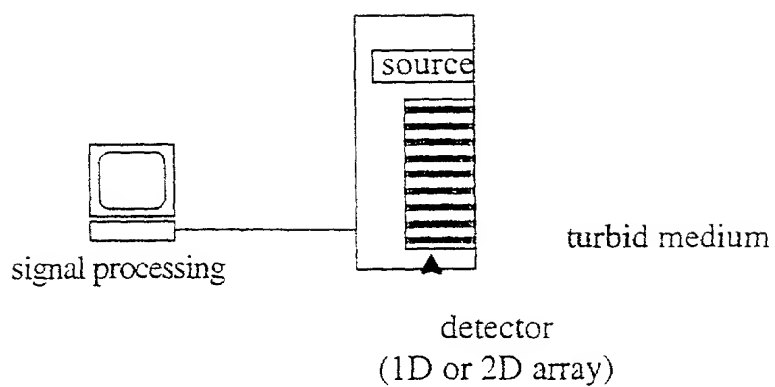
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Fig. 5.b

Apparatus:Details of the optical probe:

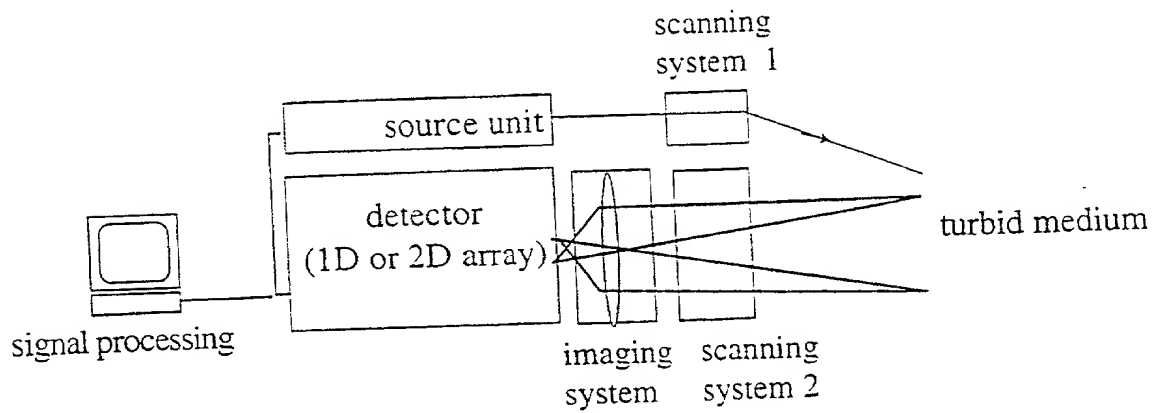
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Fig. 5c



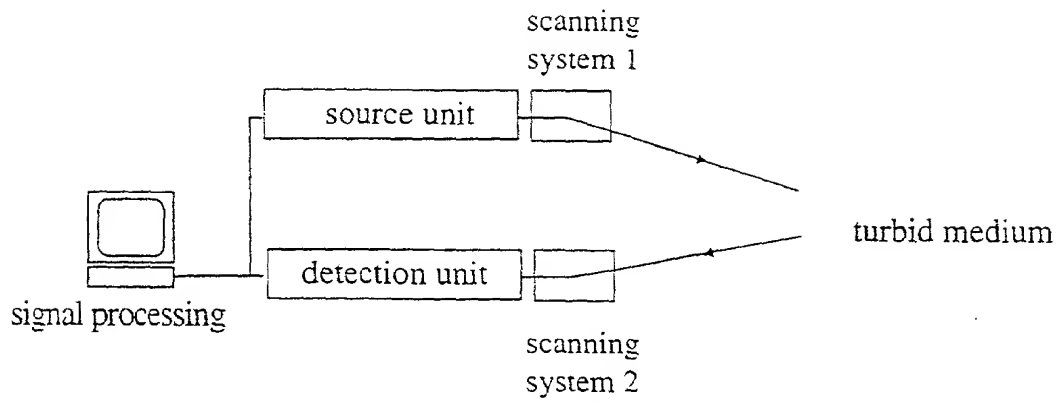
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Fig. 5d











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Fig. 5e





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 detecting fibers
 illuminating fibers
 or
 illuminating fibers
 detecting fibers

 detecting fibers
 illuminating fibers
 or
 illuminating fibers
 detecting fibers

 detecting fibers
 illuminating fibers
 or
 illuminating fibers
 detecting fibers

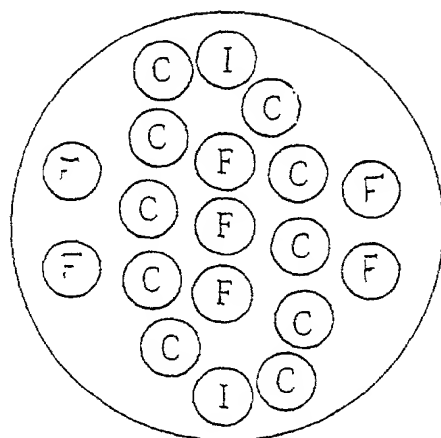
 single core fiber
(detection or illumination)

 multicore fiber or
bundle of optical fibers
(detection or illumination)

Parameter	Value	Unit
Temperature	25.0	°C
Pressure	1.0	atm
Flow rate	1.0	L/min
Wavelength	254	nm
Scan rate	10	nm/min
Integration time	1.0	s
Injection volume	10	μL
Mobile phase	Water	
Stationary phase	C18	
Column length	150	mm
Column diameter	4.6	mm
Particle size	5	μm
Retention time	12.5	min
Peak area	1234567	
Peak height	123456	
Peak width	12345	
Peak symmetry	1.234	
Peak resolution	1.234	
Peak purity	1.234	
Peak identification	1.234	
Peak classification	1.234	
Peak description	1.234	
Peak comment	1.234	
Peak status	1.234	
Peak quality	1.234	
Peak confidence	1.234	
Peak reliability	1.234	
Peak accuracy	1.234	
Peak precision	1.234	
Peak repeatability	1.234	
Peak stability	1.234	
Peak robustness	1.234	
Peak sensitivity	1.234	
Peak specificity	1.234	
Peak selectivity	1.234	
Peak linearity	1.234	
Peak range	1.234	
Peak limit	1.234	
Peak threshold	1.234	
Peak noise	1.234	
Peak baseline	1.234	
Peak drift	1.234	
Peak offset	1.234	
Peak shift	1.234	
Peak skew	1.234	
Peak tailing	1.234	
Peak fronting	1.234	
Peak broadening	1.234	
Peak narrowing	1.234	
Peak splitting	1.234	
Peak merging	1.234	
Peak co-elution	1.234	
Peak separation	1.234	
Peak resolution	1.234	
Peak selectivity	1.234	
Peak specificity	1.234	
Peak sensitivity	1.234	
Peak accuracy	1.234	
Peak precision	1.234	
Peak repeatability	1.234	
Peak stability	1.234	
Peak robustness	1.234	
Peak linearity	1.234	
Peak range	1.234	
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Peak baseline	1.234	
Peak drift	1.234	
Peak offset	1.234	
Peak shift	1.234	
Peak skew	1.234	
Peak tailing	1.234	
Peak fronting	1.234	
Peak broadening	1.234	
Peak narrowing	1.234	
Peak splitting	1.234	
Peak merging	1.234	
Peak co-elution	1.234	
Peak separation	1.234	
Peak resolution	1.234	
Peak selectivity	1.234	
Peak specificity	1.234	
Peak sensitivity	1.234	
Peak accuracy	1.234	
Peak precision	1.234	
Peak repeatability	1.234	
Peak stability	1.234	
Peak robustness	1.234	
Peak linearity	1.234	
Peak range	1.234	
Peak limit	1.234	
Peak threshold	1.234	
Peak noise	1.234	
Peak baseline	1.234	
Peak drift	1.234	
Peak offset	1.234	
Peak shift	1.234	
Peak skew	1.234	
Peak tailing	1.234	
Peak fronting	1.234	
Peak broadening	1.234	
Peak narrowing	1.234	
Peak splitting	1.234	
Peak merging	1.234	
Peak co-elution	1.234	
Peak separation	1.234	
Peak resolution	1.234	
Peak selectivity	1.234	
Peak specificity	1.234	
Peak sensitivity	1.234	
Peak accuracy	1.234	
Peak precision	1.234	
Peak repeatability	1.234	
Peak stability	1.234	
Peak robustness	1.234	
Peak linearity	1.234	
Peak range	1.234	
Peak limit	1.234	
Peak threshold	1.234	
Peak noise	1.234	
Peak baseline	1.234	
Peak drift	1.234	
Peak offset	1.234	
Peak shift	1.234	
Peak skew	1.234	
Peak tailing	1.234	
Peak fronting	1.234	
Peak broadening	1.234	
Peak narrowing	1.234	

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Fig. 6e



I: Illuminating fibers

C: detecting fibers

F: additional fibers

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Fig. 7

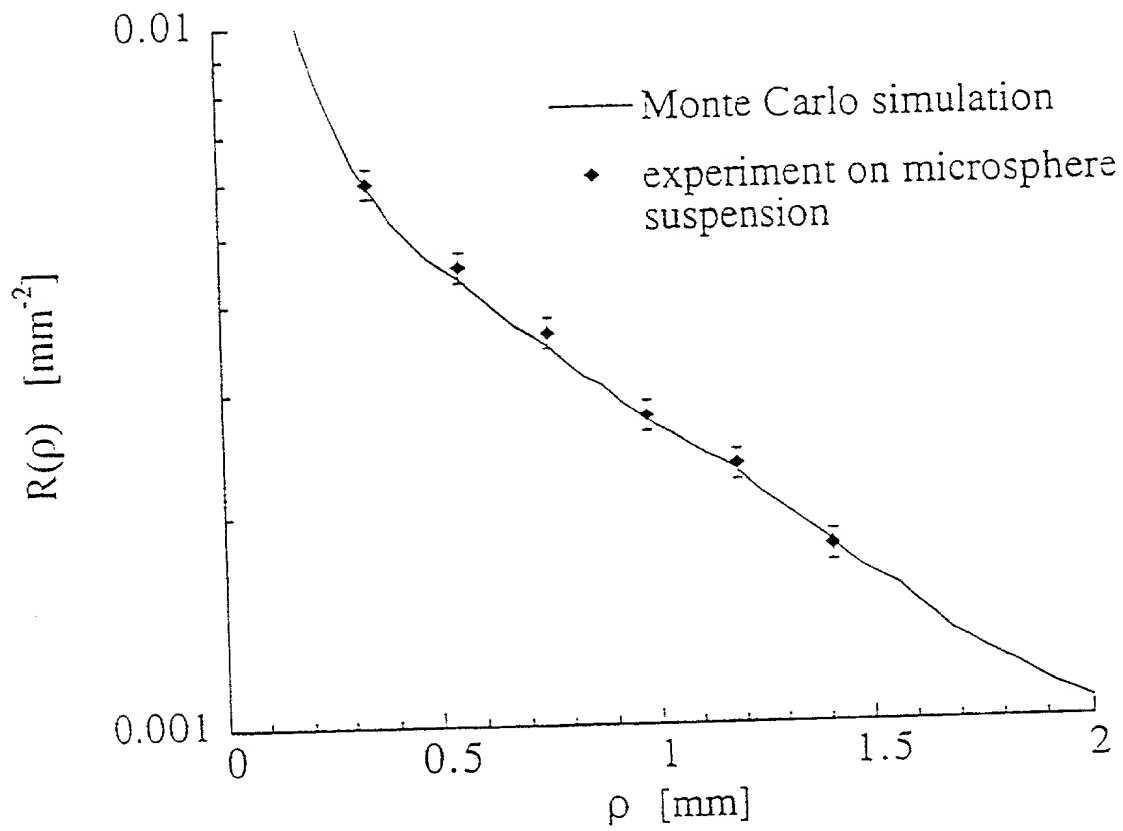


Fig. 8

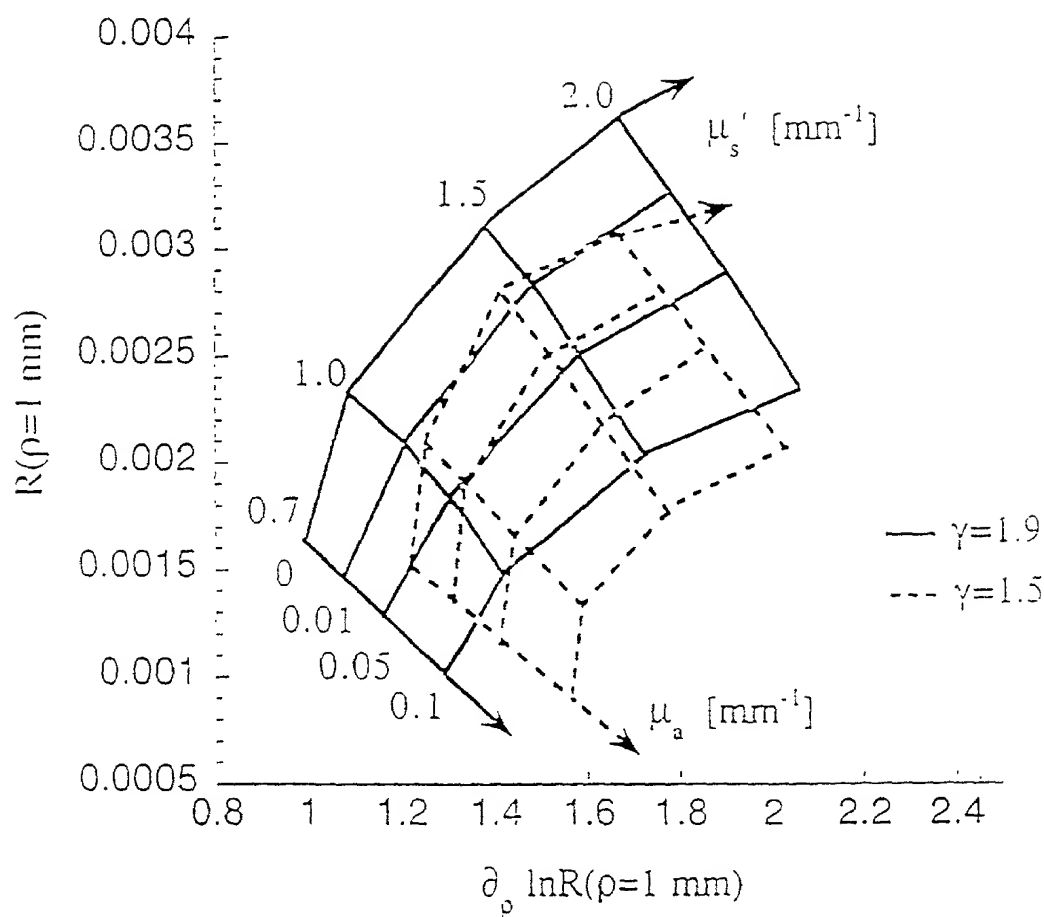


Fig. 9

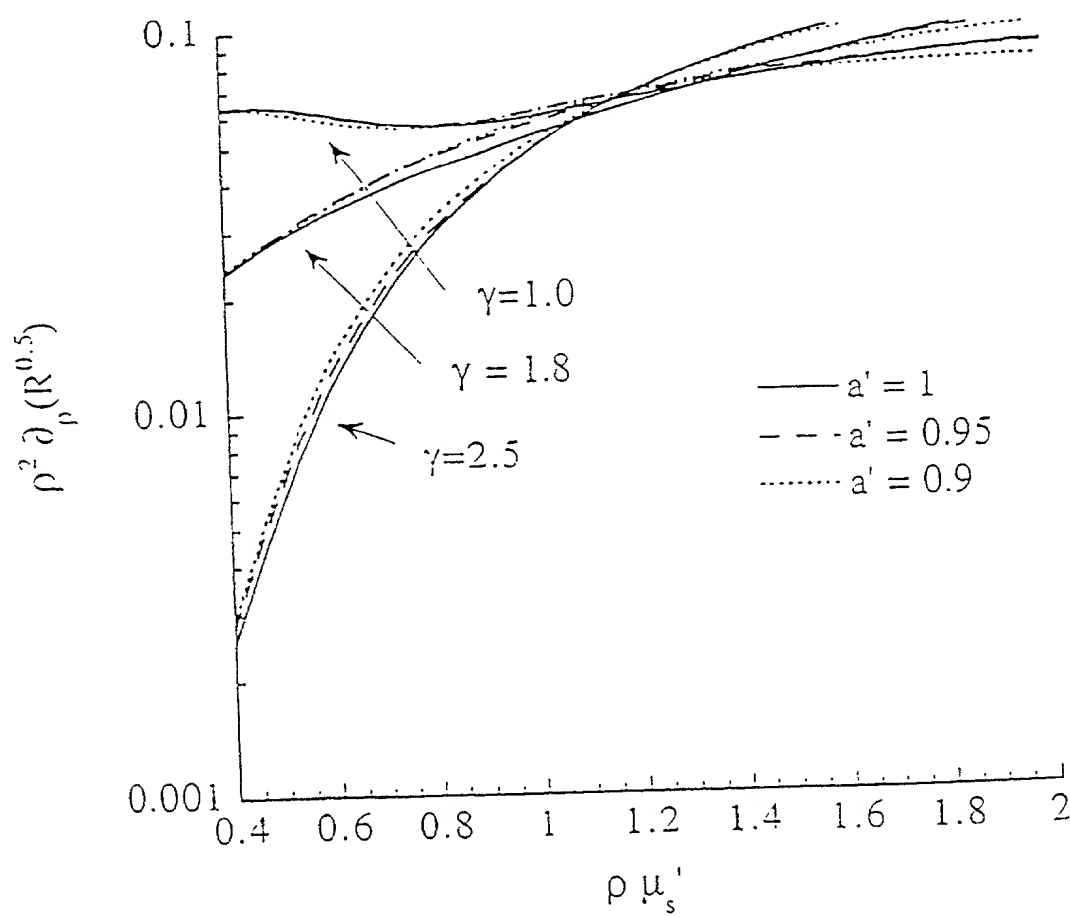
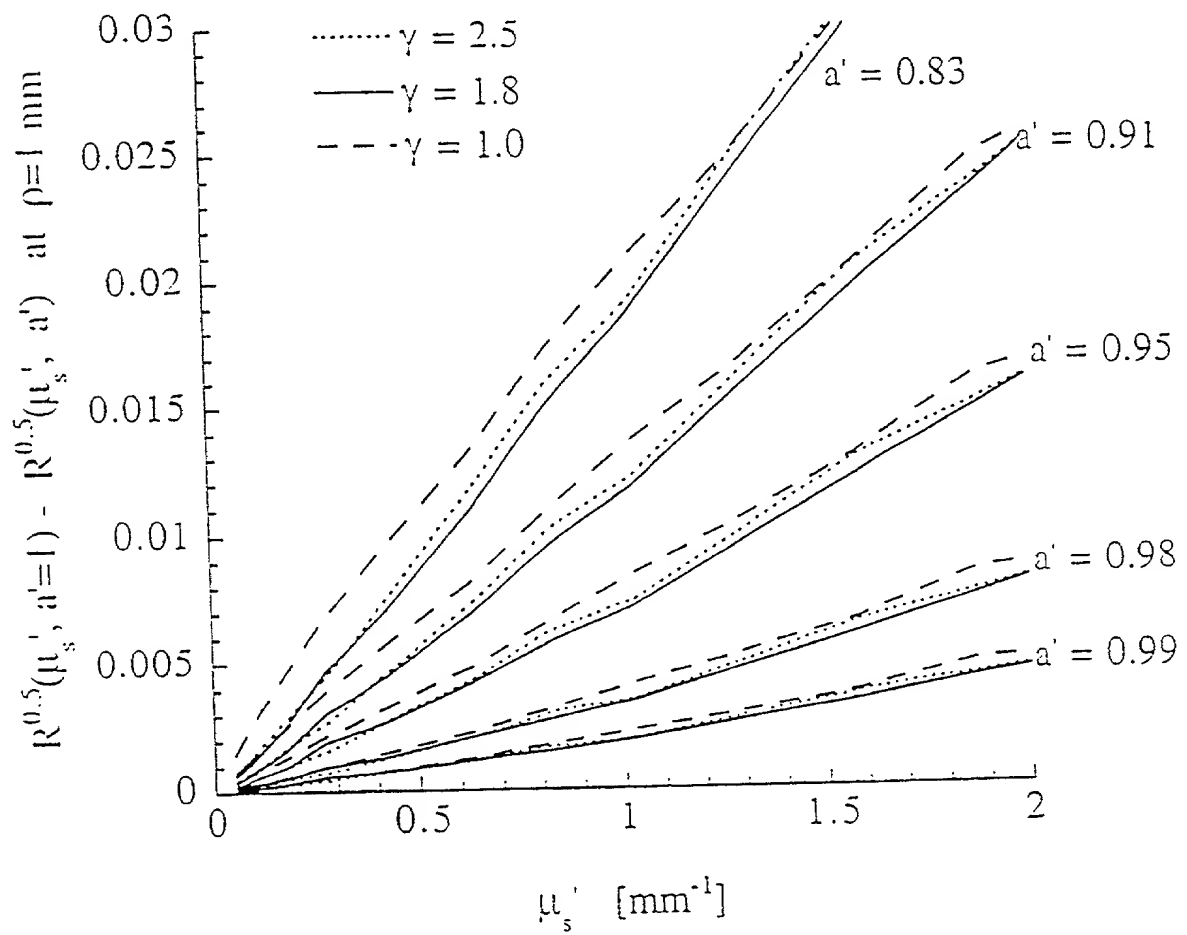


Fig. 10



RULE 63 (37 C.F.R. 1.63)
INVENTORS DECLARATION FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, mailing address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD AND APPARATUS FOR MEASURING LOCALLY AND SUPERFICIALLY THE SCATTERING AND ABSORPTION PROPERTIES OF TURBID MEDIA

the specification of which (check applicable box(es))

☐ is attached hereto
☐ was filed on _____ as U.S. Application Serial No. _____ (Atty Dkt. No. 2590-30)
☒ was filed as PCT International application No. PCT/CH99/00476 on 7 October 1999
 and (if applicable to U.S. or PCT application) was amended on 21 November 2000

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application.

Priority Foreign Application(s).

Application Number
60/103,559

Country
US

Day/Month/Year Filed
7 October 1998

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number

Date/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT International applications listed above or below:

Prior U.S./PCT Application(s):

Application Serial No.
PCT/CH99/00476

Day/Month/Year Filed
7 October 1999

Status: patented
pending, abandoned
Pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And on behalf of the owner(s) hereof, I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys thereof (of the same address) individually and collectively owner's/owners' attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Larry S. Nixon, 25640; Arthur R. Crawford, 25327; James T. Hosmer, 30184; Robert W. Farris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29008; Duane M. Byers, 33363; Jeffrey H. Nelson, 30481; John R. Lastova, 33148; H. Warren Burnam, Jr. 29366; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 38178; Robert A. Molan, 29834; B. J. Sadoff, 38863; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329; Joseph A. Rhoads, 37515; Raymond Y. Mah, 41426; Chris Comuntzis, 31097. I also authorize Nixon & Vanderhye to delete any attorney names/numbers no longer with the firm and to act and rely solely on instructions directly communicated from the person, assignee, attorney, firm, or other organization sending instructions to Nixon & Vanderhye on behalf of the owner(s).

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